

JOURNAL
OF
MORPHOLOGY

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VOLUME 32
MARCH, JUNE, SEPTEMBER
1919

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
PHILADELPHIA

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STUDIES IN THE DEVELOPMENT OF THE OPOSSUM DIDELPHYS VIRGINIANA L.

III. DESCRIPTION OF NEW MATERIAL ON MATURATION, CLEAVAGE AND ENTODERM FORMATION

IV. THE BILAMINAR BLASTOCYST

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III. DESCRIPTION OF NEW MATERIAL ON MATURATION, CLEAVAGE, AND ENTODERM FORMATION

INTRODUCTION

a. Prefatory remarks

The writer's work on the development of the opossum began in 1912-13, when a preliminary study of the problem was made and the approximate breeding season determined for Austin, Texas. Active collecting was done in January and February, 1914, and again in 1915, and the results of the study of the 415 eggs secured from twenty females were published in March, 1916. A considerable number of eggs, including several missing

stages, were also collected during 1916, and at this time many more eggs and embryos were sacrificed for a series of physiological experiments on the female opossum. As a result of these experiments I learned a simple and comparatively certain means of diagnosing a female opossum in the earliest stages of pregnancy and in early oestrus. Since it was felt that this experience would greatly facilitate collecting in 1917, plans were made to secure a complete series of eggs, embryos, and pouch young of this species. The more than hoped for success of the effort was due to the active interest of Dr. M. J. Greenman, Director of The Wistar Institute, for it was through the generosity of the Institute that I was enabled to secure and care for the requisite number of animals and also to have the advantage of the able services of Dr. C. H. Heuser, embryologist at the Institute, who, with the assistance of Miss Aimée Vanneman, technician in the School of Zoology, the University of Texas, saw to the proper fixation and after-treatment of the specimens. Entire credit also belongs to Doctor Heuser for the unique series of photographs of living eggs, some of which are herein reproduced. To Dr. J. T. Patterson is due the initiation of the work on this interesting marsupial, and his scientific zeal and keen interest in mammalian embryology have been a constant inspiration to the writer. I am indebted for indispensable assistance in the operations on the animals during the last two years' collecting to a number of premedical students of zoology, notably to Mr. Victor Tucker, who stood ready to help at any hour of the day or night and who performed many of the operations; also to Miss Janoch and Messrs. Goff, Stiefel, and Kaliski.

During the year 1917-18 I have enjoyed the privileges of a fellowship at The Wistar Institute and have been its guest while engaged in a study of the material collected. I am further indebted through the Institute to Dr. C. H. Heuser for making some of my best preparations of serial sections and to Mr. T. H. Bleakney, artist at the Institute, for drawing plate 12 and for shading and finishing the figures drawn for this paper.

The new material collected since the publication of my former paper covers many stages there described, and in addition

thereto transitional stages not secured before. Among the latter are litters Nos. 194', 344, 349, 356, 175', 339, and 347, which show the process of entoderm formation in an unbroken series. Since this phase of the problem had to be entirely rewritten, and since I now have new material on the early stages, besides a series of photographs of the living egg in all stages, it has seemed desirable and profitable to give a complete account of the development of the opossum from the beginning. This has been done in the present paper; but the reader is referred to the writer's former publication for certain details.

The original notes and the preparations upon which this work is based, together with alcoholic specimens, will be deposited in the archives of The Wistar Institute, where they will be easy of access, and anyone who wishes to do so may examine the material and test the validity of the conclusions at which I have arrived in this paper.

b. Historical

In my former publication I reviewed in some detail the work of Selenka ('87) on the opossum and that of Hill ('10) on *Das-yurus*. Mention was also made of Caldwell's discovery ('87) of the shell membrane enveloping the marsupial egg (*Phascolarctus*), and of a short paper by Professor Minot ('11) on the bilaminar blastocyst of the opossum. Simultaneous with the publication of my article, a paper by Spurgeon and Brooks appeared, giving a description of two litters of opossum eggs in cleavage (2 to 8 cells). I wish at this point to recur briefly only to the work of Selenka, leaving the other articles to be discussed under appropriate headings in the body of the paper.

Salenka's work on the cleavage and blastocyst formation is based on 26 eggs secured from two females. One animal yielded one 2-celled, one 20-celled, and nine unfertilized eggs, all badly shrunken. I suspect that the '2-celled' and the '20-celled' eggs are probably specimens in different stages of fragmentation. The other animal furnished two unfertilized eggs, one 4-celled and one 8-celled egg, two blastocysts of 42 and 68 cells, respectively, two slightly older blastocysts with a mass of entodermal cells,

and six normal vesicles with thin, partly bilaminar walls. Of his unsegmented ova I think all were unfertilized. Hence the 42-celled and the 68-celled blastocysts which Selenka describes are the youngest of his specimens which approach a normal opossum egg. These two are practically normal except for the shrinkage of the vesicle from the vitelline membrane and for the regular gradation in size of the blastomeres from one pole to the other—a condition entirely accidental and not at all characteristic for this or any other stage in the development of the opossum. His pear-shaped, thick-walled vesicle with spreading entoderm (Selenka, '87, Fig. 1 and 2, Taf. XVIII) is clearly a degenerating specimen, as I judge by comparison with numbers of similar preparations from my collection. Whenever, in any batch of eggs, there are very retarded specimens, these are to be regarded with suspicion. Many such abnormal eggs can be seen in my photographs of living eggs, as, for example in figure 4, plate 1, and figures 3 and 4, plate 11. Selenka's interpretation of certain gaps in the walls of his young blastocysts as the 'blastopore' must be rejected for the reason that these gaps are not to be found in completed blastocysts, of which I have a hundred specimens. Where openings in the blastocyst wall do exist in young specimens, they are easily explained when the method of blastocyst formation is understood.

On the origin of the entoderm in the opossum Selenka is not clear. I must support one of his suggestions, however, for his designation 'Urentodermzelle' as applied to the large cell in his 42- and 68-celled eggs expresses its true function. I previously described the rather constant occurrence of such cells, all in an excellent state of preservation; but in the absence of the succeeding transitional stages, I rejected the view that these are true entoderm mother cells and considered them of 'nomorphological importance.' I am now enabled to give a complete account of the most interesting behavior and the destiny of these cells.

On the time relations in the development of the opossum my data substantiates Selenka's account only in regard to the time between copulation and parturition, which is thirteen days.

But the ages given for all his early stages are far too low, because the author greatly overestimated the postovulatory period, that is the interval between copulation and ovulation, which he states to be five days. The time of beginning of cleavage he fixes at "exactly five times 24 hours," a period which he apparently determines on the basis of one experiment in which he secured what I regard as fragmenting eggs in a condition that accords very well with eggs about three days old. Again, his 10-hour vesicle is nearer three days old and his 32-hour vesicle nearer four days; hence the interval of twenty-two hours between these two stages is substantially correct. In a subsequent paper I shall discuss these time relations from the abundant, though by no means simple and harmonious data on hand.

c. Material and technique

1. *Material.* The opossum eggs on which the present study is based represents collections made during four seasons. In 1914 eighteen litters or batches of fertile eggs were secured; in 1915, seventeen litters; in 1916, fifteen litters, and in 1917, 37 litters—a total of 87 litters. These refer, of course, only to stages coming within the field of this paper, for besides these many litters of older stages were collected; and unfertilized eggs were removed ad nauseam. The 87 fertile litters, which include eggs through the bilaminar stage, contained 1009 eggs, of which 641, or nearly two-thirds, are normal. Thus, about one-third of the eggs secured from pregnant females are unfertilized or abnormal, chiefly the former. My previous estimate of one-sixth is therefore too low. The average number per litter is 11.5, the extremes are 1 (No. 94) and 22 (No. 346'), not taking into consideration No. 117', which numbered 43 eggs by virtue of the compensatory hypertrophy of the ovary. Table 1 summarizes the number of eggs mentioned under "History of the Animals" in the next section.

2. *Animals used; reference to illustrations.* In the following summary a brief protocol is presented of each animal furnishing eggs used in the present study. The data for Nos. 21 to 144

TABLE 1
Summary of eggs

	NUMBER OF LITTERS	NUMBER OF NORMAL EGGS	NUMBER OF UNFERTILIZED OR ABNORMAL EGGS	TOTAL NUMBER OF EGGS
A. Previously reported: litters Nos. 21 to 144, 21 different animals				
1. From pregnant animals.....	35	248	130	378
2. From pseudopregnant animals.....	2	0	37	37
B. New material: litters Nos. 173 to 415, 45 different animals				
3. From pregnant animals at first operation (left uterus).....	22	166 (63% normal)	97	263
4. From second operation (right uterus) eggs used for this article.....	14	107 (57.5% normal)	79	186
5. Later stages mentioned in summary, second operation.....	16	137 (78.4% normal)	39	176
6. From pregnant animals, proportion estimated.....	16	120 (Estimated)	62	182
7. From pseudopregnant animals.....	15	0	156	156
Total items 1, 3, 4, and 6.....	87	641 (63.6% normal)	368	1009
Total mentioned in summary.....	120	778	600	1378

are abstracted from the writer's previous study (Hartman, '16), to which the reader is referred for further details.

In the system which I have employed for the identification of the specimens each animal receives a number, and the litters of eggs taken from that animal receive the same number. Without further designation, a number may represent all of the eggs secured from both uteri when the animal is merely killed and both uteri removed simultaneously; but when the animal was used for two stages, the simple number represents the first batch

of eggs, that is, the contents of the left uterus, removed under anesthesia and aseptic conditions. The litter of eggs removed from the right uterus at a later period is designated by the prime of the number given to the animal. Thus, figure 1 in plate 1 shows the eggs No. 320 taken from the left uterus of animal No. 320 at 9 P.M., Jan. 24; figure 2 shows the eggs yielded by the right uterus of the same animal 5½ days later, and these are designated as No. 320'. The same system applies to 299 and 299', 292 and 292', etc. The first litter of eggs is invariably from the left, the second from the right uterus.

For the reader's convenience references are made to the figures illustrating the respective litters of eggs. An asterisk (*) is placed after the figure or plate containing heliotype illustrations of eggs photographed in Ringer's solution in the living state.

Animals used in the study. No. 21. Killed three days after attempted copulation; mature ovarian eggs (fig. 1, pl. 14).

No. 28. Captured Aug. 23, when seven or eight months old; kept in solitary confinement until Jan. 23, when she was placed with a male; male almost killed by female Jan. 26, indicating that oestrus had passed. Killed Jan. 27; large undischarged follicles with ripe eggs (fig. 1, pl. 13).

No. 40. Blastocysts near end of entoderm formation with greatly attenuated non-formative area (figs. 3 and 4, pl. 18).

No. 43. Eggs 0.8 to 1 mm., blastocysts bilaminar throughout (figs. 4 and 4A, pl. 20), except several like those of No. 40 (fig. 1, pl. 18).

No. 46. 2- to 5-celled eggs (text fig. 4, P).

No. 50. Unilaminar vesicles of about 50 to 70 cells with none or with one to several entodermal mother cells (figs. 1 and 3, pl. 7; fig. 11, pl. 13; figs. 2 and 5, pl. 16).

No. 52. From pronuclear to 4-celled stages, but mostly unsegmented eggs (fig. 21, pl. 14).

No. 54. About same as preceding.

No. 55. Numerous bilaminar blastocysts about 1 mm. in diam. (figs. 2, 2A, and 6, pl. 21).

No. 56. Unfertilized tubal ova still devoid of albumen layer (fig. 3, pl. 13; figs. 7 and 14, pl. 14).

No. 58. Undivided, unfertilized uterine eggs.

No. 76. Tubal ova with small trace of albumen (fig. 2, pl. 13; figs. 8, 11, 13, and 15 to 17, pl. 14).

No. 81. Fertile eggs, all 4-celled (text fig. 4, N).

No. 82. Bilaminar blastocysts like those of No. 50 (figs. 1 and 1A, pl. 20).

No. 83. Four 4-celled eggs (figs. 8, 11, and 12, pl. 15) and three young blastocysts¹ (figs 12 and 19, pl. 16).

No. 85. Cleavage stages; one each of 6, 7, 9, 10, 12, 14, 15, 17, and 18 cells; three of 8 cells; five of 16 cells (figs. 15 and 17, pl. 15).

No. 88. Of these eggs the collection contains twenty-seven excellent preparations consisting of 50 to 70 cells and ranging up to 103 cells each. Most of the eggs have from one to several entoderm mother cells in their earliest proliferation (figs. 2, 4, and 6, pl. 7; figs. 3, 7 to 11, 18, 21, and 22, pl. 16; fig. 13, pl. 22; compare also page 36, Hartman, '16).

No. 94. A single bilaminar blastocyst about 1 mm. in diameter.

No. 112. Degenerating ova from known second oestrus period.

No. 117'. Forty-three eggs, mostly in cleavage, 2- to 16-celled, from a single uterus, the organs on the opposite having been removed 33 days before; ovary hypertrophied; eggs subnormal in size (figs. 9 and 10, pl. 15).

No. 144. Blastocysts more advanced than those of No. 88; attenuation of non-formative area well under way (figs. 1 to 3, pl. 17).



Fig. 1. Three blastocysts and one unfertilized egg of litter No. 175', sketched with the aid of the camera lucida immediately upon immersion in the fixing fluid (aceto-osmic-bichromate). $\times 8$.

No. 173. Received Jan. 17. Left uterus and ovary removed 8 p.m., Jan. 18; about 12 eggs, of which 8 were sectioned: 7 are 4-celled (fig. 5, pl. 3) and one is 3-celled (text fig. 4, L; fig. 3, pl. 15).

No. 173'. At 8 p.m., Jan. 19 (interval 24 hours) about 12 just completed blastocysts were secured from right uterus; no entodermal mother cells present (fig. 5, pl. 7). Killed Feb. 9, when the completely hysterectomized, semi-spayed animal was again coming into heat.

No. 175. Received Jan. 17; removed left ovary and uterus; pseudo-pregnant; the degenerating eggs were not counted or preserved.

No. 175'. Removed male Feb. 9; killed Feb. 14 (interval 28 days after operation); 14 eggs: 6 unfertilized, 8 very attenuated vesicles, entoderm reaching almost to equator (fig. 8, pl. 18; figs. 7 and 7A, pl. 19 and accompanying text fig. 1). The measurements of the eggs of litter 175' are here given as made in salt solution:

¹ This is the only instance in all of my records in which the eggs, all removed at the same time from the animal, consisted of two distinct groups or stages, separated by a considerable period of development, in this case about twenty-four hours. There is, of course, a possibility of error on my part due to mixing of labels in this case.

Through shell.....	0.76	0.74	0.72		0.70	0.70		0.70	0.68	0.64
Through blastocyst..	0.54	0.42	0.55 x 0.4		0.50	0.44 x 0.4		0.4	0.47	0.40

No. 189. Received Jan. 22; operation at 10:45 A.M., Jan. 23; 10 eggs: 3 unfertilized; 7 bilaminar blastocysts, mostly about 1.2 mm. in diameter; one 0.9 mm. with smaller vesicle probable in dying state (fig. 18, pl. 13; fig. 4, pl. 21).

No. 189'. Killed at 10:30 P.M. same day (interval $12\frac{1}{2}$ hours); 12 eggs, five of which measured 1.7, 1.7, 1.8, 1.8, 1.9 mm.; stages just preceding the beginning of mesoderm formation; no record of unfertilized eggs (fig. 20, pl. 13; figs. 4, 4A, 7, 8, 8A, 11, 12A, 12B, pl. 22).

No. 191. Jan. 23, 3:30 P.M., took out left ovary and uterus; 10 eggs: one a defective 16-celled stage, others just completed blastocysts of about 35 cells; recorded measurements in salt solution average 0.56 mm. through shell membrane and 0.14 to 0.15 mm. through ovum (fig. 10, pl. 13; fig. 1, pl. 16).

No. 191'. Jan. 26, 9 P.M., removed right uterus, leaving ovary (interval 3 days, $5\frac{1}{2}$ hours); 11 eggs: 4 bilaminar blastocysts, 1.4 mm. in diam. in alcohol, almost no albumen; 7 unfertilized eggs. Animal died Feb. 5; no wound infection.

No. 192. Operated Jan. 23, 4:30 P.M.; 12 eggs: 4 unfertilized; 8 bilaminar blastocysts measuring mostly about 1 mm. in alcohol, but three measure 0.85, 0.90, and 1.20 mm., respectively. In xylol four measurements were 1, 1, 1.01, and 1.06, with formative areas 0.62, 0.67, 0.76, and 0.65 mm., respectively.

No. 192'. Killed Jan. 24, 11:30 A.M. (interval 19 hours); 15 eggs: 3 unfertilized; 12 vesicles, of which two measure 1.6 and 2 mm., the others about 2.4 mm.; pear-shaped embryonic area with primitive streak.

No. 193. Left uterus and ovary removed Jan. 23, 6 P.M. Number of eggs not recorded; collection contains 10 poorly fixed preparations, mostly of small blastocysts of 25 to 36 cells, one egg, however, in the 14-celled stage with two cells in telophase (fig. 8, pl. 3; fig. 9, pl. 13).

No. 193'. Removed remaining uterus Jan. 26, 8:45 P.M. (interval 3 days, $2\frac{3}{4}$ hours); number of eggs not recorded; five measured in salt solution 1.15, 1.60, 1.70, 1.70, 1.85 mm.; the first two are bilaminar blastocysts; the last three have primitive streaks in pear-shaped areas; one 1-mm. blastocyst was dead and one of 1.40 mm. has imperfect embryonic area; several unfertilized eggs (figs. 1 and 2, pl. 10; figs. 1, 9, 9A, 9B, 9C, pl. 22). Animal died Jan. 29 of an intestinal disease common in cage animals.

No. 194. Jan. 24, 8:30 P.M., found 7 young degenerating eggs like those shown in figure 6, plate 11, in left uterus which was removed with the left ovary.

No. 194'. Feb. 9, signs of approaching oestrus returned; Feb. 13, 10 A.M., copulation observed; killed Feb. 17, 25 days after first operation; 18 eggs: 9 unfertilized; 9 vesicles with entoderm only at embryonic area, stage intermediate between Nos. 356 and 352 (fig. 5,

pl. 12, figs. 13, 14, 15, pl. 17 and accompanying text fig. 2). Measurements in salt solution and in fixing fluid are as follows:

In Ringer's solution (average 0.66 mm. and 0.34 mm.).

Through shell.....	0.73	0.68	0.65	0.65	0.65	0.60	0.65	0.65
Through blastocyst.....	0.35	0.35	0.40	0.37	0.35	0.30	0.32	0.30

In fixing fluid (average 0.57 mm. and 0.33 mm.).

	<i>Hill's fluid</i>					<i>Aceto-osm.-biochr</i>			
Through shell.....	0.70	0.55	0.53	0.53	0.60	0.50	0.60	0.55	0.54
Through blastocyst.....	0.34	0.32	0.33	0.31	0.33	0.34	0.32	0.35	0.34

No. 203. Received Jan. 26. Removed only left uterus, leaving ovary, Jan. 28, 8:40 a.m.; about 12 eggs: one with pronuclei (fig. 20, pl. 14; some 2-celled (text fig. 4, E to J; fig. 7, pl. 13; fig. 1, pl. 15); one 3-celled (text fig. 4, M); others 4-celled (fig. 8, pl. 13; fig. 7, pl. 15); one recorded measurement of whole egg is 0.44 mm. through shell membrane, 0.15 through ovum.



Fig. 2. Five blastocysts with embryonic areas and one unfertilized egg of litter No. 194', sketched alive in Ringer's solution with the aid of the camera lucida. $\times 8$.

No. 203'. Second operation at 11:45; date not recorded in protocol, but cage record indicates that the time was 11:45 p.m., Jan. 29; hence the interval was probably 39 hours; removed only right uterus, leaving both ovaries. Several young vesicles of about 50 cells; one measurement in salt solution is 0.5 mm. through shell membrane, 0.16 mm. through ovum. Killed Feb. 16; corpora lutea had almost entirely disappeared, follicles still small, but mammae very thick as in pregnancy.

No. 205. Captured by dogs Jan. 28, the skin being ripped at shoulder; operated Jan. 29, 9:15 p.m.; 10 eggs: 9 young bilaminar blastocysts with much albumen at one pole; entoderm quite or nearly reaching non-formative pole; three measured in salt solution 1.05 mm. three 1 mm., two 0.90 mm., and one 0.75 mm.; one unfertilized egg measured 0.72 mm.; in alcohol after two years the eggs measured about 0.70 mm. (figs. 1, 2, 9, 11, and 12, pl. 19).

No. 205'. Killed Jan. 30, 10:10 a.m. (interval 13 hours); 13 eggs: 3 unfertilized, the remainder vesicles with faint primitive streak in rounded areas or with more advanced primitive streaks in pear-shaped areas. Two of the former measured in alcohol 1.45 and 1.83 mm. with areas 1.1 and 1.2, respectively; one of the latter 2 mm. with area 1.32 \times 1 mm.

No. 208. Caught Jan. 29; Jan. 30, 11:30 A.M., removed left uterus containing 4 eggs: 3 unfertilized, measuring in alcohol 1.1 mm., and one young bilaminar blastocyst measuring in salt solution 0.85 mm., in alcohol 0.8 mm.; size of vesicle in salt 0.65 x 0.6 x 0.5 mm. (figs. 10, 10A, and 10B, pl. 19).

No. 208'. Killed Jan. 31, 1:45 P.M. (interval $26\frac{1}{4}$ hours); right uterus yielded 8 eggs: one unfertilized, one defective vesicle, 1.25 mm. in diameter, and others like No. 205', measuring in salt solution 1.30, 1.45, 1.59, 1.59, 1.94, 2.35 mm.

No. 214 (D. marsupialis). Received from south Texas, Feb. 1; operated Feb. 2; a dozen or more undivided, unfertilized eggs, a slight degeneration apparent only after sectioning.

No. 214'. Feb. 6, right uterus removed, leaving right ovary; 14 large eggs with opaque shell membrane, dense albumen and fragmenting ova (interval 4 days). Killed Feb. 28; after 22 days the completely hysterectomized and semi-spayed animal had again come into heat.

No. 256. Removed three pouch young Feb. 9; 10 days later, numerous small eggs in early stage of degeneration were found in uteri.

No. 285. Caught Jan. 12; injured. Jan. 13, 10:25 P.M., 10 eggs: 2 unfertilized, the remainder small blastocysts partially lined with ento-

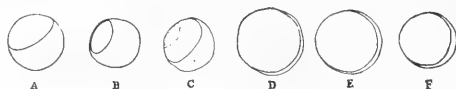


Fig. 3. A, B, and C, three eggs of litter No. 285; D, E, and F, three eggs of litter No. 285', sketched alive in Ringer's solution with the aid of the camera lucida. $\times 8$.

derm; eggs measured 0.85 to 0.9 mm. in salt solution; no preparations made of this litter (text fig. 3, A to C).

No. 285'. Killed Jan. 14, 12:30 P.M. (interval 14 hours); 14 eggs: 3 unfertilized; others as illustrated by D, E, and F, text figure 3; entodermal lining complete (least advanced, figs. 3, 3A, and 3B, pl. 20; most advanced in figs. 7 and 7A, pl. 21).

No. 287. Jan. 15, 8:15 A.M.; 7 or more eggs (the collection contains 7 preparations) undivided, unfertilized, little or no signs of disintegration (fig. 6, pl. 13; fig. 19, pl. 14).

No. 287'. Jan. 18, 6 P.M. (interval nearly $3\frac{1}{2}$ days); 13 clear, hyaline eggs, disintegration evident in ovum.

No. 290. Copulation during night of Jan. 11 to 12; motile two-headed spermatozoa recovered from vagina A.M., Jan. 12; Jan. 17, 8:45 P.M., 5 eggs: one unfertilized; 2 with small thick-walled vesicles at one pole, abnormal (compare No. 290 (3), fig. 2, pl. 6); 2 eggs with normal bilaminar vesicles occupying about one-half of the egg (compare 290 (4), fig. 2, pl. 6; fig. 6, pl. 12).

No. 290'. Killed Jan. 18, 4:30 P.M. (interval $19\frac{3}{4}$ hours); 8 eggs: 2 unfertilized; one retarded blastocyst; 5 apparently normal bilaminar

blastocysts a little over 1 mm. in diameter; no preparations made of this litter (figs. 2 and 3, pl. 11*).

No. 292. Caught with male in hollow log Jan. 13; isolated till operation, Jan. 17, 11 P.M.; 10 eggs: of the 4 that were sectioned one is a defective 7-celled egg, the others normal blastocysts of 40 to 50 cells with none or only one entodermal mother cell (fig. 5, pl. 1*; figs. 1 2, 5*, and 6, pl. 6).

No. 292'. Killed Jan. 21, 10:40 P.M. (interval 4 days); 7 vesicles about 3 mm. in diameter, late primitive-streak stage; one unfertilized egg; one degenerating young bilaminar blastocyst (fig. 6, pl. 1*).

No. 293. Caught Jan. 17; Jan. 18, 8:00 P.M., 13 eggs: of the eight preparations made all are 4-celled except one 2-celled egg; in one case one blastomere, in two cases 2 blastomeres are in mitosis (fig. 1, pl. 2*; figs. 5 and 6, pl. 15).

No. 293'. Killed Jan. 22, 7:30 A.M. (interval $3\frac{1}{2}$ days); 17 eggs: 8 unfertilized, one defective; 8 bilaminar blastocysts like those sketched in text figure 3, D, E, F (fig. 2, pl. 2*).

No. 294. Caught Jan. 17; large skin wound on belly; Jan. 18, 8:30 P.M., 15 eggs: 5 unfertilized, 8 with small rounded or irregular blastocysts at one pole, all rather abnormal; 2 apparently normal bilaminar blastocysts like F, text fig. 3 (fig. 1, pl. 11*).

No. 294'. Killed Jan. 20, 7 A.M. (interval $34\frac{1}{2}$ hours); 14 eggs: 7 unfertilized; one 1.4 mm. in diameter and 4 small degenerating blastocysts; 2 bilaminar blastocysts about 1.3 mm. in diameter, of which only one is perfectly normal. Thus both litters, 294 and 294', were mostly abnormal (fig. 4, pl. 11*). No preparations were made of this litter.

No. 298. First copulation Jan. 14, spermatozoa recovered from vagina; at 1 P.M., Jan. 15, the double spermatozoa had mostly divided. Jan. 20, 10:15 A.M., 14 eggs, of which perhaps 10 are normal blastocysts; five preparations contain 60 to 120 cells each, showing earliest entodermal proliferation (fig. 7, pl. 2*; figs. 7 and 8, pl. 6; figs. 6 and 20, pl. 16).

No. 298'. Killed Jan. 23, 12 M. (interval about $3\frac{1}{2}$ days); 6 eggs: 4 vesicles 4.25 and 4.9 mm. in diameter with medullary groove as long as primitive streak; 2 smaller vesicles and 2 unfertilized eggs (eggs in utero, fig. 8, pl. 2*).

No. 299. Caught Jan. 19; Jan. 20, 8:15 P.M., 12 eggs, of which all of the 7 sectioned are normal 4-celled eggs with small blastomeres (fig. 3, pl. 1*; figs. 6 and 7, pl. 3; figs. 13 and 14, pl. 15).

No. 299'. Killed 11:30 P.M., Jan. 24 (interval 4 days, $3\frac{1}{4}$ hours); 14 eggs: 6 apparently normal, nearly or quite completed bilaminar blastocysts; 3 abnormal blastocysts; 5 unfertilized eggs (fig. 4, pl. 1*; figs. 1 and 2, pl. 6; fig. 5, pl. 10; figs. 7 and 8, pl. 12; fig. 16, pl. 13).

No. 303. Caught Jan. 19; Jan. 20, pseudopregnant, 7 degenerating eggs a week old (fig. 7, pl. 11*); killed Feb. 1, the mammary glands still very thick, almost as in pregnancy.

No. 306. Jan. 21, 12 M.; 11 eggs recorded in notes, but only 3 found in collection; two of these are 2-celled with both blastomeres in mitosis (text fig. 4, A to D; fig. 4, pl. 3; fig. 2, pl. 15); one egg is 3-celled (text fig. 4, K; fig. 4, pl. 15).

No. 306'. Killed Jan. 26, 8:30 A.M. (interval 5 days, 20½ hours); 10 eggs: 2 unfertilized; 8 bilaminar blastocysts 0.7 to 0.75 mm. in diameter, of which one has no embryonic area (fig. 7*, pl. 10; fig. 17, pl. 13; figs. 2 and 2A, pl. 20; figs. 1 and 1A, pl. 21).

No. 307. Jan. 21, 3:30 P.M.; 11 eggs removed from left Fallopian tube (fig. 7, pl. 1*; figs. 2 to 6, 9, and 10, pl. 14).

No. 307'. Killed Jan. 27, 9:15 A.M. (interval 5¾ days); 10 eggs, unfertilized and fragmenting (fig. 8, pl. 1*).

No. 313. Caught Jan. 19; Jan. 22, 10:30 P.M., 9 tubal ova, with considerable albumen (figs. 1* and 3, pl. 3; figs. 12 and 18, pl. 14).

No. 313'. Animal died during the night; the 11 eggs taken from remaining oviduct had more albumen than the '313' litter; eggs poorly fixed (fig. 5, pl. 13).

No. 314. Copulation A.M., Jan. 20; spermatozoa recovered; Jan. 23, 7:30 P.M., 9 eggs (fig. 1*, pl. 5; fig. 1, pl. 6); of the 5 eggs sectioned one is a blastocyst of 26 cells (fig. 2, pl. 5), 2 contain about 30 cells each (fig. 4, pl. 6), and 2 are abnormal (fig. 10, pl. 21).

No. 314'. Killed Jan. 29, 10 A.M. (interval 5 days, 14½ hours); 6 normal embryos with first rudiment of allantois.

No. 318. Jan. 23, 26 eggs in early stage of fragmentation, 13 from each uterus, in which involution had already set in (fig. 6, pl. 11*).

No. 320. Received about Jan. 20; Jan. 24, 9 P.M., 13 4-celled eggs studied in salt solution; subsequent fixation poor (fig. 1, pl. 1*).

No. 320'. Jan. 30, 9:25 A.M. (interval 5½ days); 17 eggs: 4 unfertilized; 11 vesicles 2.3 to 2.6 mm. in diameter with well-developed primitive streak; one egg contains two vesicles and two embryos; two vesicles have no embryonic area (fig. 2, pl. 1*).

No. 321'. Jan. 25; litter of foetuses near term accompanied by the 4 eggs shown in fig. 9, pl. 11*; these eggs are, therefore, nearly 10 days old.

No. 332. Jan. 26, 21 eggs (9 plus 12), degenerating, unfertilized, in middle stage of pseudopregnancy (fig. 10, pl. 11*).

No. 336. Jan. 27, 9 P.M., 14 eggs (one was lost before photographing); the 6 preparations made are young blastocysts of 17, 26, 29, 30, 32, and 32 cells, respectively (all figures on plate 4*; figs. 18 and 19, pl. 15).

No. 336'. Killed Feb. 1, 5:45 P.M. (interval nearly 5 days); six 10-mm. vesicles with small embryos; 2 smaller vesicles.

No. 337. Jan. 28, 10:30 A.M., 8 eggs: a study of them in salt solution seemed to show that one egg was unfertilized, one 8-celled and six 16-celled; two eggs sectioned are 15- and 16-celled, respectively (fig. 9, pl. 1*; figs. 3* and 4*, pl. 5; fig. 16, pl. 15).

No. 337'. Feb. 1, 10:30 P.M. (interval 4½ days); 14 eggs: 12 blastocysts, about 2.5 to 4.5 mm., first appearance of medullary groove; 2 defective blastocysts (eggs in utero, fig. 10, pl. 1*).

No. 339. Jan. 28, 3:30 P.M., 8 eggs (fig. 6*, pl. 9): 2 unfertilized; 5 eggs with more or less abnormal, round, thick-walled blastocysts at one pole (fig. 2, pl. 6; fig. 15, pl. 13; figs. 5, 5A, and 6, pl. 19); one quite normal thin-walled blastocyst with entoderm spread to equator (fig. 2, pl. 6; figs. 6 and 6A, pl. 18).

No. 339'. Killed 12:30 P.M., Jan. 29 (interval 21 hours); 9 eggs: 6 bilaminar blastocysts measuring about 0.85 mm. in alcohol; one dead blastocyst 0.75 mm.; 2 unfertilized eggs (fig. 3, pl. 21).

No. 342. Received Jan. 27; Jan. 28, 9:30 P.M., 19 eggs; of the 4 sectioned specimens two are defective and the other two are blastocysts of 26 and 28 cells, respectively (figs. 6* and 7*, pl. 5; fig. 20, pl. 15).

No. 342'. Feb. 4, 8:15 P.M. (interval 7 days); 2 dead and 9 normal embryos, the latter about 7.5 mm., head-rump length.

No. 343. Observed copulation, 4 A.M., Jan. 22; Jan. 29, 2:45 P.M., left uterus yielded 15 eggs: 4 unfertilized; one small defective blastocyst; one blastocyst with defective embryonic area; 9 normal bilaminar blastocysts about 1 mm. in diameter, embryonic areas 0.64 to 0.76 mm. (fig. 5, pl. 2*; fig. 5, pl. 21).

No. 343'. Killed 7 hours, 20 minutes later ($7\frac{3}{4}$ days after copulation); 8 eggs, of which 3 are unfertilized, 5 normal 1.8-mm. blastocysts just preceding proliferation of mesoderm; embryonic areas 1 to 1.1 mm. in diameter (fig. 6, pl. 2*; fig. 2, pl. 22).

No. 344. Received and operated Jan. 29, 4:45 P.M.; 16 eggs: 15 sectioned; of these 6 are unfertilized and fragmenting; 2 are abnormal blastocysts (fig. 4, pl. 16); 7 are normal blastocysts showing early differentiation of embryonic and non-embryonic areas; the most advanced contains 124 'ectodermal' and 45 entodermal cells (figs. 5*, 6*, and 7, pl. 8; figs. 14 to 17, pl. 16).

No. 344'. Killed Feb. 1, 8:30 P.M. (interval 3 days, $3\frac{3}{4}$ hours); 7 eggs, all normal vesicles 4 mm. or more in diameter, with short medullary groove.

No. 346. Received and operated Jan. 29, 8:45 P.M.; 21 eggs: 8 unfertilized; one dead blastocyst; 8 normal 1.5 mm. blastocysts, embryonic areas about 1 mm.; the other eggs retarded and defective (fig. 3, pl. 2*).

No. 346'. Killed next morning at 6:35 o'clock (interval $9\frac{3}{4}$ hours); 22 eggs: 11 unfertilized; 11 blastocysts ranging up to 2.2 mm. in diameter, all in early primitive-streak stages (fig. 4, pl. 2*; fig. 22, pl. 13).

No. 347. Jan. 29, 9:45 P.M.; 15 eggs: 4 unfertilized; 11 normal blastocysts partly or entirely bilaminar (fig. 5*, pl. 9; figs. 5 and 5A, 7 and 7A, pl. 18; figs. 3, 8, and 8A, pl. 19).

No. 347'. Jan. 30, 10:15 P.M. (interval $12\frac{1}{2}$ hours); 17 eggs: 4 unfertilized; 13 bilaminar blastocysts measuring 1.1 to 1.24 mm. in alcohol (fig. 1, pl. 22).

No. 349. Front foot wounded in trap; Jan. 30, 3:45 P.M., 5 eggs: 2 unfertilized; one unilaminar blastocyst (fig. 4, pl. 8); 2 blastocysts with spreading entoderm (fig. 3*, pl. 8; fig. 12, pl. 17).

No. 349'. Killed Feb. 2, 11 P.M. (interval 3 days, 7 hours); 10 eggs: 9 vesicles 8 to 10 mm. in diameter with embryos of about 10 somites; one small vesicle.

No. 351. Jan. 30, 5 P.M., animal was opened; freshly burst follicles on left ovary, but no eggs in left oviduct.

No. 351'. Killed at 7:30 P.M., $2\frac{1}{2}$ hours later; 14 eggs with a little albumen found in right oviduct (fig. 2*, pl. 3; fig. 4, pl. 13).

No. 352. Jan. 30, 5 P.M.; 16 eggs: 9 unfertilized; of the 7 remaining, 3 are like eggs No. 40, the others less advanced and perhaps not quite normal (figs. 1* and 2, pl. 9; fig. 14, pl. 13; fig. 2, pl. 18; fig. 8, pl. 21).

No. 352'. Killed Jan. 31, 8 A.M. (interval 15 hours); 20 eggs: 8 unfertilized, one egg with dead vesicle; one egg with two vesicles; the remainder bilaminar vesicles fill one-half to three-quarters of the egg, which measured fresh about 0.75 mm. (fig. 4*, pl. 9; fig. 4, pl. 10; fig. 4, pl. 19).

No. 353. Jan. 30, 7:45 P.M., 16 eggs: 5 unfertilized; 11 bilaminar blastocysts measuring in alcohol 1.1 to 1.3 mm. (figs. 3, 3A, 3B, 3C, pl. 22).

No. 353'. Killed Jan. 31, 1 A.M. (interval $5\frac{1}{4}$ hours); 12 eggs: one unfertilized; one dead; 10 blastocysts 2 mm. and less in diameter, showing the proliferation of first few mesodermal cells (fig. 21, pl. 13).

No. 356. Had been in cages some time before first operation, Jan. 30, 8:45 P.M.; 15 eggs: 10 normal blastocysts averaging about 0.18 mm. through ovum, with numerous entodermal mother cells at formative pole and considerable attenuation of non-formative pole; one abnormal blastocyst with large blastomere (fig. 14, pl. 22); 4 unfertilized eggs (fig. 1, pl. 6; figs. 1* and 2, pl. 8; fig. 3, pl. 9; fig. 4, pl. 12; fig. 12, pl. 13; figs. 4 to 11, pl. 17).

No. 356'. Killed Feb. 3, 12:30 A.M. (interval 3 days, $3\frac{3}{4}$ hours); 6 vesicles about 3 mm. in diameter, with short medullary groove.

No. 360. Jan. 30, 9:30 P.M., 11 eggs: 10 bilaminar blastocysts about 1.5 mm. in diameter; one unfertilized egg (fig. 6 and stereogram, fig. 8, pl. 10; fig. 6, pl. 22).

No. 360'. Killed Feb. 2, 7:30 P.M. (interval nearly 3 days); 2 abnormal and 18 normal embryos about 5.75 mm. in length with first rudiment of allantois.

No. 415. Feb. 10, 11 fragmenting eggs in early stage of pseudo-pregnancy, presented for the false '2-celled' and '4-celled' eggs seen in figure 5, plate 11*.

3. *Material arranged according to stage of development.* The following tabulation is arranged by stages for ready reference. Within a given stage the litters are also placed in ascending order of development.

1. Ripe ovarian eggs: 21, 28.

2. Tubal ova: 56, 76, 307, 351', 313, 313'.

3. Undivided, unfertilized uterine eggs showing little or no degeneration: 58, 173, 214, 287.

4. Cleavage stages:

a. From one to about four cells: 46, 52, 54, 203, 306, 293, 81, 83, 299, 320.

b. From about 8 to 16 cells: 85, 117', 337, 342.

5. Young unilaminar blastocysts:

a. Containing from 25 to 35 cells, mostly without entodermal mother cells: 336, 173', 191, 193, 203', 314.

b. Older stages up to 100 cells, mostly with entodermal mother cells: 50, 83, 298, 292, 88.

6. Young blastocysts with distinct polar differentiation: 344, 144', 356, 349.

7. Young blastocysts with spreading entoderm: 194', 339, 352, 43, 294, 175', 347.

8. The bilaminar stage: 347, 285, 299', 205, 208, 290, 293', 43, 306', 352', 82, 285', 290', 294', 189, 191', 192, 343, 339', 94, 55, 347', 353, 346, 360, 193', 343', 189', 353' (few mesoderm cells).

9. Primitive-streak stages: 353', 346', 320', 192', 193', 205', 208', 337', 344', 356', 292', 298'.

10. Embryos: 349', 336', 314', 342', 360', 321'.

11. Unfertilized and degenerating eggs: 112, 415, 175, 194, 256, 287', 307', 214', 318, 303, 297, 332, 321'.

4. *Securing the eggs.* During the collecting season 1916 and 1917 two stages were secured from each female after the method first employed by Bischoff on the rabbit. As the method has proved of great value to the writer in securing a complete series of stages, it is here discussed in some detail.

The female is placed under anesthesia and one uterus is removed under aseptic conditions; the animal recovers and the eggs are allowed to 'incubate' in the remaining uterus for a calculated period of time. In this way, by utilizing gradually accumulating data on the rate of development, it became possible to secure almost any desired stage and thus fill in the gaps still appearing in the series. Thus, for example, I succeeded in securing from animal No. 353 eggs in which the mesoderm was

just beginning to proliferate. Animal No. 343 had previously furnished bilaminar blastocysts (fig. 5, pl. 2) from the left uterus; she was killed seven hours and twenty minutes later and a litter of large blastocysts, still in the bilaminar stage, was removed (fig. 6, pl. 2): the interval allowed had been too short. Animal No. 346 (figs. 3 and 4, pl. 2) had yielded bilaminar blastocysts a little larger than No. 343 and an interval of nine hours and forty-five minutes had proved to be too long, for, when the animal was killed, the primitive streak was already well advanced in the second litter of 'eggs. Profiting by these two experiments, when animal No. 353 appeared with large bilaminar blastocysts about the size of those in litter No. 346, a five and one-fourth hour interval proved to be the correct one, for the eggs in litter No. 353' contain the first anlage of the primitive streak, one egg having as few as twenty-five mesodermal cells.

In the operations I have found it most convenient to enter the abdomen through a short slit on one side of the pouch. For the sake of uniformity I select the left side as a matter of routine. The animal is shaved over this area and the incision is made as near the pouch as possible, care being taken not to cut through the pouch, especially in multiparae, which possess dilated pouches. The operated animal is bandaged; but it is impossible to keep the bandage on an animal unless the entire trunk is covered. I use over the bandage a jacket with holes cut for head and legs and tied over the back. As the animal usually sweats with the bandages on, the wound will heal better if they are removed at the end of three or four days.

If, on opening the animal, the uteri are purplish and flaccid, the case is one of pseudopregnancy and the organs may be left intact and the animal kept for another oestrus period, which takes place in about thirty days. If ovulation is recent, however, one uterus must be removed to ascertain the state of the eggs. If the appearance of the organs indicates that young stages are to be expected, the uterus is placed in warm Ringer's solution and a slit is made through the musculature and peritoneum from one end to the other, and this must be done by a rapid manipulation of the scissors to prevent eversion of the mucosa.

The pressure now being removed, the hypertrophied mucosa is pulled apart, preferably under the binocular microscope, with two pairs of finely pointed forceps, and the lumen exposed. The eggs may be picked out from among the delicate folds of the mucosa by means of a pipette. But this method is unnecessarily tedious; the uterus may instead be simply turned inside out in the Ringer's solution and the eggs picked out from the bottom of the dish. To insure finding all of the eggs, a little Bouin's fluid added to the salt solution, after removal of all the eggs that can be seen, makes any specimens overlooked prominently visible. The uterus should also be shaken out in another dish of Ringer's solution for any eggs that may have been hidden in the uterine folds. To keep the solution clear of blood, it is well, before opening the organ, to slit all the superficial blood-vessels and drain them of blood. I may add that the neck of the uterus should be ligated with a 'lifting' ligature before it is cut from the body, in order to prevent the loss of eggs through the os uteri.

Young eggs in cleavage and small blastocysts are mostly found near the caudal end of the uterus, often closely bunched together. Hence one cannot speak of 'implantation' of the opossum egg at any early stage. The 'uterine cups' described by Spurgeon and Brooks ('16) do not mark implantation surfaces, but merely accidental pits produced by pressure into the delicate oedematous mucosa.

If, on opening the animal, pregnancy seems to be advanced, in order to remove entire vesicles intact, it is best to slit the uterus superficially in many places and to trim off the entire musculature before attempting to remove the vesicles, which are closely applied to the mucosa, but never fused with it. This procedure renders the use of a killing fluid to paralyze the musculature entirely superfluous. With a pair of forceps and a fine brush an entire litter of delicate vesicles may be removed intact. They may be transferred to the fixing fluid in a deep mustard spoon or in a shallow, neckless vial. A collapsed vesicle may again be dilated in the fixing fluid by injection with a fine pipette; in fact it is well to irrigate with the fixing fluid the lumen of every vesicle containing a large embryo.

Eggs are easily washed out of the Fallopian tube by means of a stream of Ringer's solution, as has been done in other mammals.

5. *Fixing and staining.* I have used the following solutions: Bouin's, Bouin's half strength, increased gradually to full strength; Hill's; Flemming's; Carnoy's; Zenker's; formol-Zenker; picro-sulphuric; trichloracetic; Bensley's aceto-osmic-bichromate. Hill's fluid is made as follows: Mayer's piconitric, 96 cc.; 1 per cent osmic, 2 cc.; glacial acetic, 2 cc. I stated in 1916: "I have found Hill's mixture to be the perfect fixing liquid for the opossum egg." Further experience with it has led me to give decided preference to Bouin's for all older blastocysts; for younger eggs up to the bilaminar stage I get equally good fixation with both; and I also have made some poor preparations with either. For all stages Bouin's is perhaps the safest solution to use; with it the specimens have the advantage of toughness and they can be safely transported, whereas solutions containing osmic acid render the specimens unduly brittle. The half-strength Bouin is not as good as full-strength. I have some excellent preparations of material fixed in Flemming's fluid, although collapse of blastocysts is more likely to occur in this fluid than in Bouin's. My poorest fixation was with aceto-osmic-bichromate, although superficially the eggs thus fixed seem well preserved. This fluid has the advantage of bringing out cell membranes clearly. I have no perfect specimens fixed in Zenker or formol-Zenker, both of which shrink the material more than any other and render it very brittle. Several fairly good preparations were made with picro-sulphuric. Trichloracetic has the peculiar property of fusing extruded yolk and cytoplasm of the blastomeres into an almost undifferentiated mass (fig. 13, pl. 15).

Hematoxylin stains, especially Heidenhain's iron-alum hematoxylin, have proved entirely satisfactory, both for sections and for surface mounts. Several eggs fixed in solutions containing osmic acid were stained in acid fuchsin, saffranin, or cochineal to differentiate the nuclei clearly from the black yolk granules, but this refinement of technique is not at all necessary.

To prevent collapse of the blastocysts, which my photographs on plates 1 and 2 show to be perfect spheres, it is important to pass from one medium to another (Bouin's to alcohol, water to alcohol, alcohol to xylol, and especially xylol to paraffin) by slow gradations. I use 5 per cent differences, accurately made up in stock solutions. The eggs are placed in small vials and each higher percentage is added gradually. Vesicles from about 1.5 mm. on up may easily be cut in half equatorially with fine scissors; but such hemispheres, if of approximately equal size, should not be placed in the same vial, because they are likely to telescope in such a way that they are hard to separate without injury to them.

The material was imbedded in paraffin and most of the newer material was sectioned by Huber's water-on-the knife method, which gives incomparably better results than ribboning the series with the rotary microtome.

6. *Unfertilized eggs.* Since, unfortunately, the vast majority of the eggs removed from animals in captivity are unfertilized, it will not be amiss to mention them here. Such eggs remain in the uterus for many days, undergoing progressive degeneration before being discharged or absorbed. For the first two or three days they are not easily distinguished from normal uterine eggs. The first sign of degeneration is the breaking up of the ovum into masses of various shapes and sizes (fig. 5, pl. 11) and the ovum may flatten out into the shape of a crescent (fig. 6, pl. 11). Gradually, too, the eggs increase in opacity (fig. 7, pl. 11) and become covered with white concretions (fig. 8, pl. 1; figs. 8 to 10, pl. 11), so that they are only too prominent when one opens the uterus hoping to find embryos. The eggs shown in figure 9, plate 11, accompanied fetuses near term; hence these eggs are at least nine days old.

7. *The illustrations.* The drawings (plates 12 to 22) were made on Ross stipple board No. 2 and reduced to one-sixth the original size. Korn's lithocrayon No. 1 (a paraffin-carbon pencil) gave the best results, since to be reproduced by the line process the dots must be absolutely black, a result not easily attained with a graphite pencil.

The drawings on plate 12 were made free hand by Mr. T. H. Bleakney from stained specimens in oil of wintergreen, the size being calculated from photographs of the living eggs. The eggs shown in text figure 4 were mostly drawn from wax models made after the Born method, $\times 600$. This figure was also drawn on Ross board No. 2, but was reproduced one-third of the original size. All other drawings, except a few where especially mentioned, were made from sections and were drawn as nearly like the specimens as possible, imperfections and all. The attempt was made to reproduce not only the form, but also the texture of the specimens, and for this purpose the Ross board has proved to be a delicate and responsive medium.

The smaller drawings were outlined with the camera lucida at a magnification of $\times 300$, $\times 1200$, $\times 3000$ (reduced to $\times 50$, $\times 200$, $\times 500$); the larger sections of blastocysts, since they had to be drawn at a magnification of 1200 which resulted in drawings more than a meter long, were first sketched $\times 400$ with a Leitz-Edinger drawing apparatus, then photographed and the negative finally projected to the desired magnification by the Edinger apparatus. To facilitate measurements, the scale of a stage micrometer was drawn beside the first sketch made and appears upon the negative made from it.

The photomicrographs were made with Spencer lenses, which afford a flatter field for photographing than the Zeiss microscope lenses. Attention is especially directed to the photographs of living eggs. More than 500 different eggs are represented in the heliotypes. All of the photographs on plates 1, 2, and 11 and many other figures are magnified eight times and some are at a higher magnification.

The photographs are unretouched and are reproduced as exactly like the original as was possible with the process employed.

To secure an absolutely black background for the photographs taken by reflected light, we found it best to use a black watch-glass. The eggs are removed from the uterus and placed in a deep watch crystal in clear Ringer's solution free from dirt and blood-cells. The watch crystal is now set into the watch-glass

which must also be filled with the solution; for it is absolutely essential that there should be no air space for the reflection of light between the transparent glass holding the eggs and the black glass serving as a background.

In the photographs of preparations, as well as in the drawings, for ease of comparison, magnifications of 50, 200, and 500 have been adhered to with few exceptions. In this connection plates 12 and 13 are especially adapted to serve as a résumé of the stages covered in this paper. Comparison of these two plates shows that the young eggs shrink greatly on account of their delicate albumen layer.

Altogether the twenty-two plates accompanying this paper contain over 750 representations of more than 600 different opossum eggs, mostly, of course, in groups on the photographs. The drawings and the photomicrographs of preparations number some 240 of over 180 different eggs.

d. External changes at ovulation in the female opossum

In common with many other wild animals, the opossum does not breed well in captivity. I have worked with hundreds of animals kept in cages or in large rooms, isolated or in groups of dozens or of a hundred or more; yet the number of observed copulations that I have to record is disappointingly small. Many births have taken place in the cages, but the cases of pseudopregnancy outnumber the cases of true pregnancy many times over.

In spite of careful personal attention to the habits of the captive animals, I was unable during the first two years' collecting to determine from outward signs the sexual state of the female. In this regard I was at first forced to agree with Selenka who says: "Ohne operative Eingriffe ist über die Trächtigkeit eines Weibchens keine Gewissheit zu erlangen, da man weder durch Tasten mit dem Finger die weichen Uterushörner auffinden kann, noch auch an den Milchdrüsen eine Veränderung wahrnimmt, bevor nicht die Embryonen nahezu ausgewachsen sind." I have since learned, however, that Selenka was wrong

in his statement concerning the mammary glands. For, during the 1916 season, I found that by simple palpation of the mammary glands within the pouch I was able to diagnose with a high degree of accuracy the state of the internal reproductive organs, so responsive are the glands to the physiological changes going on in the animal just before and after oestrus. By this method one is enabled to select from the animals on hand those that are likely to furnish eggs or embryos. Thus out of the hundred animals Nos. 300 to 400, used at the height of the breeding in 1917, only a half-dozen failures are recorded. A typical case of misjudgment is that of No. 326, in which '5-mm. vesicles' were predicted and ripe follicles found in the ovary; or No. 354 in which 'bilaminar blastocysts' were expected and the animal was found in pro-oestrus. Sometimes a later stage than the one predicted will be found, as is, of course, to be expected from individual variations that are general in all physiological processes. The method has resulted in the saving of a great deal of time, effort, and material, especially during the last breeding season.

Unfortunately, however, the physiological changes which the mammary and other reproductive organs undergo are identical immediately after oestrus whether pregnancy ensues or not. This holds true for the mammary glands more than for the other organs, and it is impossible during the first five or six days to distinguish externally between pseudopregnancy and pregnancy. As ovulation is always spontaneous, the internal organs behave the same in both conditions. The vaginal loops begin to retrogress even before ovulation. The uteri are almost maximum in size when the minute eggs first reach them; in pregnancy they remain bright red and turgid and possess a peculiar luster like polished red agate; but, if the eggs are unfertilized, the uteri, after four or five days, become dull and dark red and then flaccid and collapsed. The corpora lutea are somewhat more persistent in true pregnancy. But the mammary glands continue development even after the degeneration of the corpora and the involution of the uteri are well under way.

c. Are the eggs of operated animals normal?

The question may well be asked whether we are dealing with normal material in the case of eggs removed some time after an abdominal operation under anesthesia or whether such treatment of the mother affects the development of the eggs unfavorably. It should be emphasized at the outset, however, that whatever answer we give to the question does not affect the conclusions reached in this study, which is supported by an abundance of material from freshly killed animals and by a large assortment of specimens removed from animals at the first operation. It should also be noted that the time interval between the two operations was in many cases only a few hours or a half-day, so that in this material, too, the chances of modifying the normal course of development of the eggs were reduced to a minimum. From a careful examination of my notes and a scrutiny of both classes of material I have concluded that there is no evidence pointing to deleterious effects of the operation, and I here present some of the facts that have led me to this conclusion.

In the first place, the condition of the operated animals was as good or better than that of non-operated cage animals; for the former were the choice specimens, vigorous in health and sexually active. As stated above, I am now able to determine, with a high degree of accuracy, the near approach of oestrus in the female opossum. A surprisingly large number of females are captured (and by the terms of our contract with the hunters must be purchased) which are either too old or too sick to breed. Specimens with deep, infected wounds, intestinal diseases, xerophthalmia (McCollum), or other nutritional disturbances do not come into heat. Only once or twice have I seen females with badly infected wounds continue in the oestrus cycle like a normal animal; but I have records of dozens of cases in which the normal sexual processes were interrupted by wounds or disease during pro-oestrus or dioestrus. Pregnant females, however, pass successfully through the period of gestation even in

the same condition that prevents the onset of oestrus. On the other hand, operated animals recover quickly, often eating heartily several hours after the operation. Their wounds heal readily and the animal comes into heat again even after two operations and after double hysterectomy, in the same manner as if the abdomen had not been opened. Certainly, if Bischoff a century ago was able to secure as many as six different stages of normal embryos from one rabbit, without anesthesia or asepsis, successively opening the abdomen and ligating off segments of the uterine horns "until inflammation set in," then a very simple operation on the opossum under modern surgical precautions should have no deleterious influence on the embryos.

If, now, to test the matter further, we compare the proportion of normal eggs secured at the first operation (table 1 above) with the proportion from the second operation, we find 63.1 per cent normal (item 3, table 1) for the former and for the latter 67.4 per cent (items 4 and 5, table 1), an unexpectedly but quite accidentally large percentage of normality for the operated animals.

These figures, however, include under 'abnormal' all unfertilized eggs, which should be left out of consideration, since we are here testing the effect on the development of the eggs and embryos. We must, therefore, count only the dead and defective fertilized eggs in given litters, selecting comparable stages. Table 2 gives this data for bilaminar vesicles of litters in which every egg was studied; and cases from the second operation are selected in which at least one day had intervened after the first operation. Table 3 gives similar data for primitive-streak stages up to 5 mm. in diameter. The litters are arranged more or less in order of relative stage of advancement.

From a study of tables 2 and 3 it is apparent that there is a high rate of mortality in the eggs of the opossum, both of operated and unoperated animals, but that wholly normal litters occur in both classes. Some cases are of special interest. No. 334, for example, yielded a perfect litter of eggs from the left uterus, but only a single abnormal vesicle 20 hours later from the right uterus. On the other hand, No. 344 yielded 7

normal and 2 abnormal eggs of an early stage and after an interval of three days a perfect litter of 7 normal vesicles. Litter No. 339 from the left uterus is largely abnormal, whereas No. 339' from the second operation was largely normal. Both batches of eggs from animal No. 294 were mostly abnormal, possibly on account of a temporary interference with the circulation of the uteri when the animal was twisted out of its lair

TABLE 2
Number of abnormal eggs at the bilaminar stage

LITTER NUMBER	INTERVAL	NUMBER OF NORMAL EGGS	NUMBER OF ABNORMAL EGGS	TOTAL NUMBER OF FERTILIZED EGGS
a. At first operation				
	<i>days</i>			
339	0	1	5	6
205	0	8	1	9
343	0	9	2	11
346	0	8	5	13
353	0	11	0	11
360	0	10	1	11
Total.....		47	14 23%	61
b. At second operation				
299'	4	6	3	9
293'	3½	8	1	9
339'	1	6	1	7
306'	4¾	7	1	8
191'	3¼	4	0	4
Total.....		33	6 10.8%	37

after a method employed by hunters and applied in this case. Litters No. 175' and No. 194', twenty-five and twenty-eight days, respectively, after the removal of the left uterus, contained no abnormal eggs.

Later embryos and fetuses present facts similar to those just indicated for the younger stages. To refer to special cases shown in table 4, No. 349, which had furnished only 3 normal

eggs out of 5 from the left uterus, had 9 large, normal embryos and one dead embryo in the right uterus three and one-half days later, and this in spite of the fact that the animal was unusually small and had one of its legs wounded in a trap. But of the six large embryos yielded by the left uterus of No. 379, an apparently normal female, only one was normal; but 18

TABLE 3
Number of abnormal eggs at primitive-streak stages

LITTER NUMBER	INTERVAL	NUMBER OF NORMAL EGGS	NUMBER OF ABNORMAL EGGS	TOTAL NUMBER OF FERTILIZED EGGS
a. At first operation				
180	0	5	0	5
338	0	14	4	18
380	0	8	2	10
334	0	11	1	11
211	0	10	1	11
211'	12½ hours	8	0	8
Total.....		56	8 11%	63
b. At second operation				
	<i>days</i>			
208'	1	7	1	8
292'	4	6	1	7
320'	5½	11	2	13
337'	4½	10	2	12
344'	3	7	0	7
356'	3	6	0	6
298'	3½	3	3	6
180'	1	11	1	12
334'	1	0	1	1
Total.....		61	11 15.3%	71

hours later all of the 6 embryos in the other uterus proved to be normal.

As such cases could be multiplied, the facts are that there is a mortality in the opossum ovum at all stages and that the death

rate is not affected by the abdominal operations such as employed in our experiments.

TABLE 4
Number of abnormal embryos of later stages

LITTER NUMBER	INTERVAL	NUMBER OF NORMAL EMBRYOS	NUMBER OF ABNORMAL EMBRYOS	TOTAL NUMBER OF EMBRYOS
207	0	11	1	12
207'	2½ days	3	7	10
226 (left)	0	3	4	7
226 (right)	0	0	all	
291	0	10	0	10
291'	½ day	7	0	7
379	0	2	4	6
379'	18 hours	6	0	6
314'	5½ days	6	0	6
336'	5 days	6	2	8
342'	7 days	9	2	11
349'	3½ days	9	1	10
360'	3 days	18	2	20

MATURATION AND CLEAVAGE TO THE FORMATION OF THE BLASTOCYST

a. The ripe ovarian egg

Since the publication of my former paper I have not seen the first maturation spindle, for the ova of all large follicles thus far studied in numerous series of ovaries have either germinal vesicles in the resting stage just preceding maturation or have already given off the first polar body. Recently collected ovaries containing large follicles are now being prepared and will be discussed in connection with a paper on the corpus luteum.

The ripe ovarian egg is broadly elliptical in form, but may be nearly spherical, as some dissected specimens indicate. Measurements were previously stated to average 0.165 x 0.135 mm. or larger than any tubal or uterine ova. This is large in comparison with Eutherian ova, but small in comparison with

the egg of *Dasyurus*, which measures 0.21×0.126 to 0.27×0.26 mm. The ova shown in figure 1, plate 13, and in figure 1, plate 14, are unusually large, even for ovarian eggs, measuring 0.183×0.156 mm. (average 0.175 mm.) and 0.185×0.15 (average 0.167) mm., respectively. That the ovarian ova are on the average larger than the tubal or the uterine ova would seem to be the case from the few measurements of ovarian eggs that have been made. There is, of course, considerable variation in the sizes of different eggs, both of the same litter and of different litters (fig. 2, pl. 3, figs. 2 to 6, pl. 13).

The ova are surrounded by a well-defined zona pellucida, within which the polar body is found. This is given off usually at one of the ends of the somewhat elongated egg (fig. 1, pl. 13), but it may be found near the equator. The polar body is small and flattened, and contains chromatin matter and a minimum of cytoplasm. The chromosomes of the egg nucleus lie in the cytoplasm near the polar body, mostly more or less discrete and arranged in an equatorial plate. In this condition the egg reaches and traverses the Fallopian tube.

The ovarian egg is, therefore, essentially like the tubal ovum presently to be described in greater detail. There is no polar differentiation recognizable except for the location of the polar body. It is important to note also that the yolk has no tendency to accumulate at one pole of the egg, as is so strikingly the case in the mature ovum of *Dasyurus* and to a slight degree in certain Eutherian eggs (bat, armadillo). Herein lies the first striking difference between the eggs of *Didelphys* and the Australian *Dasyurus*.

b. The tubal ovum

1. *Material, ovulation, secretion of albumen and shell membrane.* Eggs were removed from the Fallopian tubes of five animals, but in no case was insemination observed. Unfortunately, none of the thirty or more eggs sectioned contains any trace of a spermatozoon. This stage has, therefore, not yet been observed in the case of any marsupial.

Eggs were found in both tubes of female No. 56; and, since both batches of eggs had practically no albumen deposited on their surface, they must have been discharged simultaneously from both ovaries a short time before their removal. None of these eggs possesses any granulosa cells nor was any semblance of a 'corona radiata' ever observed on any tubal ova. Their naked condition when discharged is positive evidence that Selenka ('87) was in error when he considered the shell membrane of uterine eggs as the modified granulosa cells—an error made despite Caldwell's correct interpretation of this structure.

Litter No. 76 was taken from one Fallopian tube only, the opposite one not yet having received the ova, which had, however, been discharged from the ovary on that side, as indicated by the presence upon it of fresh corpora lutea. I infer that the eggs must have been lost in the body cavity. Since the eggs secured from the right uterus had already been provided with a small quantity of albumen, one may assume that they had anticipated the eggs from the other ovary by a short space of time.

No. 351 had ovulated at 5 P.M., when the animal was first opened, but no eggs were found in the left Fallopian tube, they having also been lost in the handling of the organs. Two and a half hours later the right tube contained eggs which had a distinct albumen layer on all sides (fig. 2, pl. 3). If this represent the amount of albumen deposited in two and a half hours, it would require twenty-four hours for the eggs to traverse the Fallopian tube and receive their entire quantum of albumen. Professor Hill thinks that the eggs of *Dasyurus* pass through the tube very quickly, since he has never found a whole litter of eggs in the tube. But the uterine eggs of *Dasyurus* are very scantily provided with albumen; in fact, never relatively more than the incomplete deposit around the eggs of my litter No. 351 (fig. 2, pl. 3).

Eggs No. 307 (fig. 7, pl. 1) show a greater deposit of albumen on one side of the egg. Since this is true to some extent in all the eggs found in the upper part of the tube, and since, later,

the albumen is of about the same thickness on all sides (fig. 1, pl. 3), the eggs are probably rolled about slowly as they pass through the Fallopian tube.

The shell membrane is doubtless secreted and added to the surface of the albumen in the lower part of the oviduct. Insemination must of necessity take place soon after the eggs enter the tube before albumen is deposited; for spermatozoa are found in some eggs throughout the albumen and most often nearest the ovum. The eggs of litters Nos. 336 and 356 have enormous numbers of spermatozoa entangled among the lamellae of the albumen; in figure 12, plate 13, for example, the spermatozoa are seen to occur in thick clusters as well as scattered singly throughout the albumen.

Usually an ovum is necessary to afford the stimulus for the secretion of the albumen; but in one case a rounded mass of epithelial cells proved adequate, and there was produced a structure without an ovum, the cell mass replacing the latter in the center of the egg. Epithelial cells from the wall of the Fallopian tube, enmeshed within the albumen, are of common occurrence. In another case an ovum and a cell mass and in a third case two ova were included within the same egg envelopes. Both of these latter ova would be likely to develop, if one may judge from the cases of double ova in the blastocyst stage shown in figure 2, plate 1, and in figure 4, plate 9. An egg of a parasitic roundworm once found among tubal ova did not seem to afford the adequate stimulus for the secretion of albumen.

2. Size and shape. The ova vary greatly in size and shape, not only among the different litters, but also among the eggs of a single litter. They are elliptical, rarely spherical in shape, as may be seen from the figures in plates 3 and 14. The average size of thirty-one preparations on the slide is 0.122×0.104 (av. 0.113) mm. Twelve eggs of litter No. 56 average 0.128×0.109 (av. 0.118) mm.; this list includes two whole mounts which are nearly round and measure 0.131 mm. (fig. 7, pl. 14) and 0.135 mm., respectively, the latter being the largest tubal ovum in the collection. The maximum length of elliptical eggs of this

batch is 0.142 mm., the maximum width 0.120 mm. The average of eight eggs of batch No. 76 is 0.134×0.113 (av. 0.123) mm.; the maximum length is 0.148 and maximum width 0.125 mm. But this batch includes also two very small eggs measuring 0.100×0.085 (av. 0.093) mm. and 0.090×0.083 (av. 0.087) mm., the latter being the smallest egg in the collection. Three eggs of batch No. 307 average 0.126×0.116 (av. 0.121) mm. in the preparations; the size of the fresh eggs of this batch, shown in figure 7, plate 1, cannot be given because the magnification of the photograph is not known. The two living eggs of batch No. 313 shown in figure 1, plate 3 measure 0.119×0.105 (av. 0.112) mm. and 0.106, respectively; the average of eleven eggs of batch No. 313', as photographed in the living state, is 0.108×0.099 (av. 0.103). In the preparations, three eggs of batch No. 313 measure 0.105×0.091 (av. 0.098) mm., indicating some shrinkage of the eggs in the histological processes. Batch No. 351' (fig. 2, pl. 3) average 0.110×0.096 (av. 0.104) mm. in the living state; three preparations from this batch measure 15 per cent less, namely, 0.102×0.076 (av. 0.089).

3. *The distribution of yolk.* The opossum egg, in common with the eggs of other marsupials, is rich in yolk or other lipoid deposit, which partly accounts for their larger size (figures on pl. 14). The fat occurs in the form of granules or spherules, many or perhaps all of which stain black with osmic acid. Eggs fixed in Bouin's fluid show numerous vacuoles from which the fat is dissolved in clearing. The fat content of the eggs renders them much less transparent; but in the living state the globules may be seen in the egg and they also appear distinct near their outer limits of distribution in the photographic negatives taken by transmitted light in salt solution. Thus the negative from which figure 1, plate 3, was made shows in detail oil globules quite similar in distribution to those shown in figures 12 and 18, plate 14. A study of the fresh eggs and photographs of them convinces me that the fixed and sectioned specimens accurately show the true details of these eggs, little altered by the histological processes.

Three more or less distinct regions are typically recognizable in many of the ova (fig. 12, pl. 14). There is a marginal zone, sometimes very narrow, consisting of granular cytoplasm, nearly devoid of fat granules. Beneath this is a more or less diffuse zone of oil globules, which may be very small or very large or medium in size, as seen from the figures on plate 14. Some litters show considerable uniformity in this respect (No. 76) and in others there is variation within the litter (No. 313, figs. 12 and 18, pl. 14). The outer surface of this zone of fat globules is often marked by a delicate region which may break down in fixation (light zone in figs. 12 and 19), reminding one of the delicate deutoplasmic pole of the egg of *Dasyurus*. In the living opossum egg this region is a light band interrupted here and there with oil globules coming near the surface. The third region is the large central portion of the egg which is rather uniformly granular and contains few oil globules or vacuoles.

The tubal ovum, like the ovarian ovum, exhibits no polar concentration of yolk, which is in striking contrast to the unsegmented ovum of *Dasyurus*, in which the deutoplasm is gathered in a mass at the vegetative pole of the egg and is bodily extruded just prior to the first cleavage; whereas in the opossum the yolk is thrown out from both ends or from all sides in greater or less amounts, as the sequel will show.

4. *The polar body.* The first polar body, which is present in all ripe ovarian eggs and in all tubal ova, lies in a spindle-shaped depression under the vitelline membrane. It is never large, containing a modicum of cytoplasm, in contrast to the prodigality with which yolk and cytoplasm are eliminated from the egg in cleavage. The polar body is usually of such a peculiar color and homogeneous texture that it is easily recognizable in eggs fixed in Bouin's fluid; but if the fixing fluid contain osmic acid the polar body is seldom recognizable. The chromatin is usually a deeply staining irregular mass.

Both polar bodies are soon absorbed, disappearing as distinctive structures in early cleavage. Except for a slight difference in size, the two polar bodies are practically identical. I

have seen them in numerous eggs in cleavage, especially in 4-celled eggs. The oldest stage which contains two objects that I take to be polar bodies is a blastocyst of 34 cells. The polar bodies are caught between two blastomeres of the vesicle (fig. 1, pl. 16). The larger of the two is shaped like a bent spindle and resembles in outline the space which it occupied while still crowded in the usual periovarial space before cleavage. I have never seen polar bodies so large that they appear in cross-section like those figured by Spurgeon and Brooks ('16).

5. *The chromosomes.* The spindle for the second maturation division is formed soon after the giving off of the first polar body, and in this condition the egg reaches the Fallopian tube. The vesicular or resting stage does not seem to intervene between the two maturation processes. Insemination was not observed. In the absence of spermatozoa, the ovum reaches the uterus unchanged, except for the accession of the egg envelopes. Thus the young uterine eggs Nos. 58, 287, and others have chromosomes practically indistinguishable from those about to be described for tubal ova.

Three preparations from batch No. 307 (fig. 7, pl. 1; figures on pl. 14) are especially favorable for a study of the chromosomes and for determining their number. These are still scattered along the clearly defined spindles, the equatorial plate being delayed in its formation. Some of the spindle fibers are thick and beaded as though they were derived from the fibers of the preceding division. One spindle is contained in a single section (fig. 6, pl. 14). There are clearly twelve chromosomes in each of these eggs. In all other tubal ova the chromosomes are closely arranged in a more or less definite equatorial plate and are difficult to count; but I am sure that the number is twelve in five or six cases, and I can count at least ten or eleven chromosomes with distinctness in all cases. Hence I am prepared to state that twelve is the reduced number of chromosomes in the egg of the opossum.

Figures 11 and 14, plate 14, represent the usual appearance of the chromosomes in these specimens, and in these two cases twelve chromosomes can be clearly made out. Figure 13 shows

a side view of a spindle in which eight chromosomes are seen and short fibers are clearly outlined. The three sections shown in figures 15 to 17, plate 14, were cut tangentially through the egg, hence the polar body is cut longitudinally and the chromosomes are seen in polar view.

The chromosomes in every case are short and thick, never characteristically rod-shaped. Some are hollow squares with rounded corners, others more perfectly ring-shaped. In side view several appear as short, thick rods, slightly constricted in the middle; others bent or cupped so as to appear narrowly kidney-shaped. The spindle is usually situated in a granular area free of vacuoles or fat globules; or, in other words, in the region of the spindle, the central and the marginal granular regions are bridged across. The polar body is usually placed near the chromosomes, as seen in the figures. In one case one chromosome was extruded with the polar body (fig. 8, pl. 14).

c. The young uterine egg

1. *Size and shape.* The appearance of young uterine eggs is well illustrated by the photographs in plates 1, 2, 5, 11, and others, which represent them with fidelity just as they were removed from the uterus. In size the eggs, as measured through the shell membrane, are subject to considerable variation among the different litters as well as to some extent within a given litter. Thus litter No. 342, consisting of about the 26-celled stage, average 0.7 mm., whereas the 4-celled eggs of litter No. 293 average 0.57 mm. and the eggs of litter No. 292, which are young vesicles of some 100 cells, measure 0.55 mm. Again, litters No. 336 and 337 average 0.73 and 0.50 mm., respectively, although they are in nearly the same stage of advancement and the ovum proper is of about the same size in the two litters. The differences in diameter among the eggs is therefore a difference in the quantity of albumen deposited about the ovum. Figure 1, plate 12, represents an average unsegmented uterine ovum.

2. *The albumen.* The albumen is laid down in delicate, concentric lamellae around the ovum (pl. 13 and others). In the living state it is usually of nearly the same density throughout the layer (fig. 2, pl. 4) or it may be more concentrated about the ovum in young eggs (figs. 3 and 4, pl. 5).

At first the albumen is extremely poor in protein content, for on fixation it usually gathers immediately about the ovum and the thin and delicate shell membrane collapses and follows close upon the albumen (fig. 5, pl. 5). This phenomenon is apparent on comparing plate 12, which represents the living condition, with plate 13, on which corresponding stages are shown from sections on the slide. The shrinkage of the ovum proper or the whole egg in later stages is comparatively slight; only the albumen of the younger egg suffers great collapse. It follows, therefore, that the albumen layer gradually increases its protein content (figs. 14 and 17, pl. 13), and the shell membrane likewise grows in thickness and resistance. The uterine 'milk' doubtless supplies the material thus absorbed. This holds true for unfertilized eggs also, which continue to grow in diameter and in density of albumen and shell membrane for a week or more. Figures on the thickness of the shell membrane have previously been given (Hartman, '16) and are not repeated here. It is subject to great variation, as may be seen from the various drawings in the plates, where the shell membrane is represented in correct proportions.

3. *The unsegmented ovum.* Unless insemination has taken place, the uterine differs from the tubal ovum only in the possession of completed albumen and shell envelopes (fig. 19, pl. 14). My collection contains a number of litters of such eggs. The first polar body and the second maturation spindle are as in the tubal ova, although in some case the chromosomes begin to show a clumping and are surrounded by a light area. The chromosomes fragment sooner or later, however, and the chromatin breaks up and rearranges itself into round lumps simulating nuclei in the resting stage. The cytoplasm breaks up also, some fragments taking one or several 'nuclei,' others none. Sometimes the fragments are equal or nearly equal in size, so

that such eggs may easily be taken for cleavage stages (fig. 5, pl. 11). I have never seen a mitotic figure in such degenerating eggs. The eggs shown in the photograph in figure 8, plate 1, remained in the uterus until near the end of the sixth day after ovulation.

4. *The pronuclear stage.* Several eggs were studied at this stage, although I did not secure an entire litter of eggs containing pronuclei. The pronuclei at first lie at the periphery of the ovum in a homogeneous granular area devoid of fat globules. Eventually they come to lie near the center of the egg where the first cleavage spindle will form. The chromatin of the pronucleus is in most cases very diffuse and stains weakly. Two figures of the stage are given in the writer's former paper and figure 20, plate 14, is an ovum with two nuclei which differ from the nuclei of the other members of this litter (2-, 3-, and 4-celled eggs); hence I regard this egg as in the pronuclear stage.

d. *The first cleavage*

1. *The first cleavage spindle.* No new material containing the first cleavage spindle has been obtained recently. In figure 21, plate 14, I have redrawn specimen 52 (3) as a composite of four sections, which were taken obliquely through the spindle, which lies in the central yolk-free zone of the egg. The fat vacuoles are evenly distributed at the poles and some of the yolk has already been extruded, chiefly on one side of the egg.

2. *The 2-celled stage.* The early cleavage material in the writer's collection was, however, considerably increased by recent accessions, namely, from the following litters: No. 173, which furnished 3- and 4-celled eggs; No. 203, which furnished four 2-celled, one 3-celled, and the rest 4-celled eggs; and No. 306, which furnished, besides a number of eggs that were unfortunately lost or misplaced, two 2-celled and one 3-celled egg. All of these litters are the product of the left uterus and in each case a later stage was removed from the right uterus, which is good evidence that we are here dealing with normal fertilized material.

Of the 2- and 3-celled eggs models were prepared and drawings made from the models, which are shown in text figure 4. I, J and P of this figure were drawn from eggs mounted in toto in balsam.

The blastomeres of the 2-celled eggs are usually flattened as if by mutual pressure upon their contact surfaces. They may be of equal size and shape and practically identical, or they may be unequal, as the drawings in the figure amply show. If they differ in size I rather believe this difference to be secondary and not to unequal cleavage, that is, to the greater amount of yolk extruded from the smaller blastomere. Thus, in egg No. 203 (13), shown in I, text figure 4, one blastomere has given off a large mass of yolk at each end, but the aggregate of the masses in the two halves of the egg is as nearly equal as in the adjoining figure of a sister egg.

A study of the eggs in serial section fails to reveal either a qualitative difference between the two blastomeres or the slightest indication of polarity within the blastomeres themselves (fig. 4, pl. 3; figs. 1 and 2, pl. 15). The yolk granules occur in equal numbers and sizes at the two poles and the nuclei are centrally placed. The distribution of the yolk granules is indeed quite similar to that of the undivided egg, namely, in a zone toward the margin of the cell (figs. 2 to 4, pl. 15), and this holds true also for the blastomeres of the 16-celled stage and even later (fig. 17, pl. 15). In no case is it possible to distinguish a more deutoplastic 'vegetative' pole and a relatively yolk-free 'animal' pole in any stage of segmentation.

There would seem, however, to be a qualitative difference in the blastomeres, as evidenced by the more precocious division of one of them in the formation of the 3-celled stage, which in later divisions leads to the 6- and the 12-celled conditions.

e. The second cleavage

1. *The 3-celled stage.* The 3-celled egg differs from the 2-celled stage only in the more rapid division of one of the blastomeres (figs. 3 and 4, pl. 15). In all the 3-celled eggs the

large blastomere has two nuclei (text fig. 4, K, L, M) and in one egg the cytoplasmic division is initiated, as indicated by a constriction around the cell. The interesting point in these eggs lies in the position of the blastomeres to one another, especially in eggs Nos. 306 (3) and 173 (8); for the lines joining sister nuclei are almost absolutely at right angles to each other. The usual position of the blastomeres of the 4-celled stage (text fig. 4 N to P; figures on pl. 15) is, therefore, already anticipated in the 3-celled egg. The shifting of the blastomeres in egg No. 203 (3); shown in text figure 4, M, is rather along the original plane of the 2-celled stage, and such an egg might develop a 4-celled egg like that shown at O, whereas an egg like No. 173 (8), shown at L, would be sure to develop into the typical ovum with cross-shaped blastomeres, as in figures 5 and 6, plate 15.

2. *The 4-celled stage.* If the number of specimens which the collector happens to secure of a given stage be any criterion of the relative length of time which the egg remains in that stage, then according to my collection the 4-celled condition of the opossum egg is not passed very quickly. For I have more than five dozen, mostly excellent preparations of this stage, and have other eggs still unsectioned. Three whole litters (Nos. 293, 299, 320) furnished only 4-celled eggs so far as these have been studied. However, this preponderance of 4-celled eggs is probably quite accidental. Inasmuch as cleavage proceeds irregularly after the 4-celled stage, it would not be fair to compare the number of 4-celled with the number of 8-celled eggs, for example, for a litter preponderatingly 8-celled would be sure to contain 6-, 7-, and perhaps 10- and 12-celled eggs also (compare Nos. 85, 117, 342).

The 4-celled egg of the opossum is typically Eutherian in the cross-shaped arrangement of the blastomeres. This is quite evident from the figures presented, some of which are drawn from models, others from in toto preparations and from sections (text fig. 4 and pl. 15). The arrangement of the blastomeres is such that no section can possibly pass through the centers of all the four blastomeres. If the imbedded egg be so oriented that

a section cuts two blastomeres the other two have a chance to be similarly cut (figs. 6 and 7, pl. 3; figs. 11 and 12, pl. 15). Sometimes three blastomeres are found in one section and a single one in another section (figs. 9 and 10, pl. 15). In figure 5, plate 3, and figures 7 and 8, plate 15, the knife passed through the centers of two blastomeres, the top of a third, and the bottom of the fourth. I have also studied 4-celled eggs in the living state under strong illumination and have clearly seen that the crossed arrangement of the blastomeres, sometimes with slight deviations from 180° , is normal for the opossum egg.

The four blastomeres of any one egg are usually of the same size; hence one can seldom differentiate a pair of large and a pair of small cells, and I have searched in vain for any other trace of polarity in these eggs aside from that afforded by the occasional presence of the polar bodies which, with the shifting of the cells, has little meaning (figs. 11 and 12, pl. 15). Moreover, the blastomeres are always spherical, except when very large, in which case they are flattened on contact surfaces by mutual pressure (figs. 7 and 9, pl. 15). The entire ovum measures through the zona the same as the undivided tubal or uterine egg. Among the various litters of eggs there is, however, a remarkable variation in the relative size of the blastomeres, which depends upon the amount of yolk extruded. The egg represented in figure 7, plate 15, has a minimum of eliminated yolk and the largest blastomeres; figure 14 represents the other extreme; figure 11 the intermediate condition. The extent of yolk elimination would seem to be hereditary, for in each batch of eggs the blastomeres of the individual eggs are approximately of the same size; thus, in No. 203 they are all very large, in No. 299 all extremely small. Both types are, however, normal, for sister ova in the right uterus in each case were allowed to develop and produced normal blastocysts.

The eliminated yolk in the 4-celled eggs seems to be characteristic of this stage. It occurs in small rounded lumps of about equal size, uniformly distributed (figs. 5, 6, and 7, pl. 3; figs. 7, 8, 11, 12, and 14, pl. 15).

f. The origin of the crossed arrangement of the first four blastomeres

Since in the Eutherian ova there is very little yolk to be eliminated, even in cases, such as the bat, where the phenomenon has been described by Van der Stricht, the blastomeres of the 4-celled stage fill the space within the vitelline membrane rather snugly. It has therefore been suggested that mutual pressure is responsible for the shifting of the blastomeres and that in the crossed arrangement they occupy the minimum space in the egg. A glance at the specimen photographed in figures 6 and 7, plate 3, will convince one, however, that this mechanical explanation is inadequate, for certainly here one cannot speak of mutual pressure of the blastomeres, for they are not even in contact, and yet in such eggs the shifting also takes place. Hence we must look for other causes of the shifting movement.

It is, of course, quite possible that there is no shifting at all, but that the cleavage planes cut the two blastomeres of the 2-celled egg at right angles, as has been suggested by Professor Hill ('10, p. 31). According to this assumption, one of the blastomeres would be divided meridionally, the other equatorially, and the crossed arrangement would obtain from the beginning. Indeed, a study of the 3-celled eggs described above would seem corroborative of this view, for here the definitive arrangement has already manifested itself. But two facts make this theory untenable. First, a number of 4-celled eggs and one 3-celled egg I find to deviate less than 180° from the parallel arrangement; hence for these one would under the theory have to postulate a backward shifting toward the parallel position.

But conclusive evidence on the point is furnished by eggs Nos. 306 (1) and 306 (2), in each of which both blastomeres are in mitosis. In the latter the spindles are exactly parallel, as shown by lines connecting their ends in D, text figure 4. In the former egg (A and B) the spindles in the blastomeres are 36° removed from the parallel. These observations seem to indicate that division begins in both blastomeres of the 2-celled

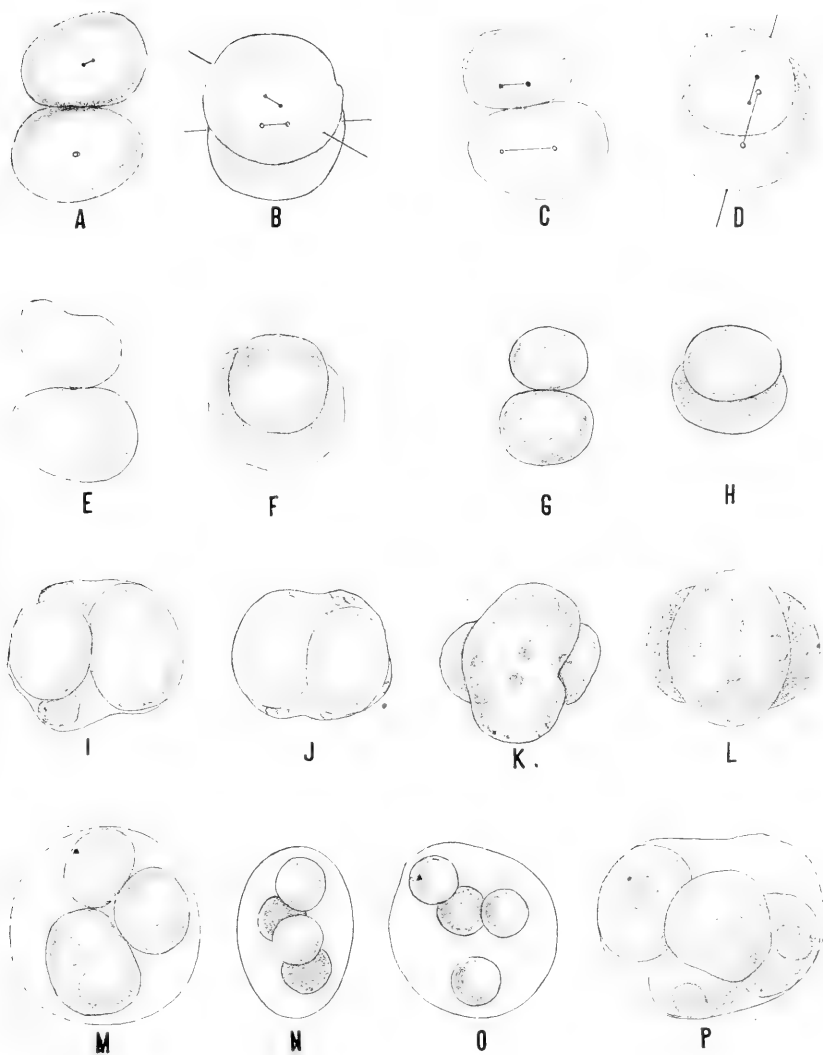


Fig. 4. A and B, two views of egg No. 306 (1); C and D, two views of egg No. 306 (2); in both cases both cells are in mitosis and the lines run through the ends of the spindles (compare fig. 2, pl. 15, and fig. 4, pl. 3). E and F, two views of egg No. 203 (8) (compare fig. 1, pl. 15). G and H, two views of egg No. 203 (4) (compare fig. 7, pl. 13). I, No. 203 (13); one blastomere only has given off a large amount of yolk. J, egg No. 203 (11). K, egg No. 306 (3) (compare fig. 4, pl. 15). L, egg No. 173 (8) (compare fig. 3, pl. 15). M, egg No. 203 (3). N, No. 81 (6). O, No. 299 (7) (compare fig. 13, pl. 15). P, No. 46 (7). I, J, and P, drawn from total preparations; all others from wax models.

egg in a single cleavage plane and that secondarily a shifting sets in early in the process of division.

There is a third possibility, as described by Sobotta ('95) for the mouse. According to this author, if I follow him correctly, one of the two blastomeres of the 2-celled egg divides meridionally, but the other blastomere has the division spindle at right angles to the first cleavage plane. In other words, the cleavage plane of the first blastomere to divide stands at right angles to the first cleavage plane, whereas in the second blastomere it is parallel to it. In such a case some shifting is also necessary to bring about the typical crossed arrangement of the 4-celled egg. This method does not obtain in the opossum, as is seen from my description above.

This point would appear to be further complicated by Spurgeon and Brooks ('16), who describe and figure cleavage stages, derived apparently from two female opossums. According to these authors, the second cleavage plane passes through both blastomeres equatorially and not meridionally, and thus a fourth method is suggested. I would cheerfully accept the authors' conclusions, but for the fact that the eggs described by them do not appear to me to represent normal fertilized eggs. I believe their specimens to be fragmenting and unfertilized eggs that have been in the uterus three or four days. My reasons are as follows: 1) Cases of fragmenting eggs are extremely common in cage animals and such eggs may fragment into regular pieces resembling blastomeres of eggs in cleavage, as I have seen repeatedly in hundreds of such eggs (compare my photograph in fig. 5, pl. 11). 2) In their illustrations some of the blastomeres have an additional peculiar nucleus and many of the nuclei are very eccentric in position. Multinucleated 'cells' and those with nuclei placed at a distance from their centers are quite characteristic of fragmenting eggs. 3) The 'polar bodies' represented are peculiar for their large area in cross-section and for their position at a distance from the periphery of the egg. 4) The authors do not figure any of their 4-celled eggs, of which they secured four along with other stages, although they present drawings of six 2-celled and other eggs. 5) The photographs

given by the authors in their figures 12, 13, and 14 I recognize from my experience with hundreds like them as typical pictures of degenerating eggs; for example, in the thickness of the shell, which suffers little collapse in fixation; in the peculiar stringy, not uniformly concentric character of the albumen, and in the character of the ovum itself, where fragmentation is quite apparent. 6) Finally, the size of the eggs as stated by the authors, 0.75 to 1.5 mm., is far above that of normal eggs in cleavage and entirely in agreement with my own specimens of fragmenting eggs. I must, therefore, conclude that the eggs described by Spurgeon and Brooks do not represent normal cleavage in the opossum.

I would conclude, therefore, that both blastomeres of the 2-celled opossum egg divide meridionally, but that they shift their position during division so that the resulting 4-celled ovum possesses the typical crossed arrangement.

g. Comparison of the 4-celled egg of the opossum and of Dasyurus

It is seen from the foregoing that the 4-celled stage of the opossum is typically Eutherian, at least in the arrangement of the blastomeres, and quite different in every recognizable way from the egg of *Dasyurus*, in which, as described in Hill's beautiful monograph, the second cleavage is shown to be meridional, dividing the egg into four equal cells which exhibit the same polar differentiation as the 2-celled egg, for each blastomere possesses a larger, vegetative pole and a smaller, relatively yolk-free animal pole.

Precisely such an egg is described by Selenka ('87) for the 4-celled stage of the opossum. I can reaffirm my former statement that this is a case of an unfertilized egg undergoing pseudo-segmentation or amitotic fragmentation, in which the four pieces or 'blastomeres' (pseudoblastomeres) happen to be of equal size. I have seen such eggs dozens of times. Figure 5, plate 11, is a photograph of a litter of eggs, palpably fragmenting, but showing one '2-celled' and one '4-celled' stage which might easily be mistaken for normal cleavage. This

matter is again mentioned and the photograph presented as further evidence that there is a decided difference between the normal 4-celled egg in the opossum and that of *Dasyurus*. What has been described by various authors as 'parthenogenetic cleavage' in ovarian eggs of mammals may often be merely a fragmentation process similar to that here described for the opossum. I have also found just such fragmenting eggs in atretic follicles of opossum ovaries.

h. Deutoplasmolysis or the elimination of yolk

In the eggs of both *Dasyurus* and the opossum the extrusion of yolk proceeds in the manner that one might predict from the distribution of the yolk in either case. In the ripe egg of *Dasyurus* the deutoplasm is collected in a mass at one pole where it is bodily extruded when the first two blastomeres round up during the first cleavage. In the opossum the yolk, being peripherally distributed, is given off from any or all sides. This happens, in small amounts, as early as the pronuclear stage and in larger amounts at the first cleavage. At each cleavage stage some yolk is left within the blastomeres, and it is probable that with each succeeding division of the blastomeres some additional yolk masses are eliminated. There seems to be no regularity of time in the elimination of the yolk, just as there is no regularity in the relative amounts eliminated; but the greatest quantity seems to be given off between the 2- and the 4-celled stage. The blastomeres may be very large and full of yolk or very small and proportionally yolk-free; and, since considerable cytoplasm is thrown off with the yolk, this would seem to indicate that a relatively unimportant rôle is played by the peripheral cytoplasm in the normal processes of the cells. But the fate of the yolk is in all cases the same: it is eventually digested and resorbed, so that in the bilaminar stage only a few granules occur among the cells of the embryonic area, as will be pointed out later.

As the eliminated material contains both cytoplasm and yolk granules, it would seem that whole portions of the cells are

dropped bodily. The appearance of these cast-off masses in the various stages may be seen from the drawings. With trichloracetic fixation, blastomeres and yolk blend into an almost uniform mass, so that the limits of the cells are recognizable with difficulty (compare fig. 13, pl. 15, with fig. 14, eggs from the same litter).

The yolk elimination in marsupials is, of course, striking in that the mass involved is very large, and this is as one would expect from the phylogenetic position of the group, as has been so ably discussed by Professor Hill. This phenomenon has, however, not entirely disappeared among the Eutheria, as Van der Stricht's fine study of the bat ovum amply proves. This author has shown that there is a polar distribution of the yolk in the bat egg and this undergoes elimination, a process called deutoplasmolysis by the author. A similar condition is found in the ovum of the armadillo by Newman ('12), but this author's statement that the similarity in the distribution of deutoplasm in the eggs of the armadillo and of *Dasyurus* argues for the low phylogenetic position of the Edentata loses some of its force from the fact that the egg of *Didelphys*, a marsupial, does not exhibit a polar concentration of fat.

i. Later cleavage to the formation of the blastocyst

An extended description of the later cleavage of the opossum egg was presented in my former article (Hartman, '16), to which the reader is referred for details here omitted. The new material collected in 1916 and 1917 contains eggs from 8 to 26, 28, and more cells; all corroborative of the former account. These eggs were also carefully studied in the living state and were photographed in salt solution at high and low magnifications, and the assurance may be given that the fixed and sectioned material accurately represents the true morphological relations. This is well borne out by the photographic reproductions of living eggs and of sections made from them as shown on plates 4 and 5.

The later cleavage is represented in the collection by eggs with every number of blastomeres from the 4-celled stage in which two blastomeres only are in mitosis (figs. 5 and 6, pl. 15) to the fully formed blastocysts of about 32 to 36 cells. Cleavage proceeds very irregularly after the 4-celled stage, which explains the fact that the 8-celled and the 16-celled eggs are only slightly in the plurality (compare litter No. 85). There is a retardation in division of cells at one pole of the egg, presumably among the lineal descendants of one of the first two blastomeres. In models of 10- and 12-celled eggs the larger cells are grouped at one pole, but, aside from this fact, there is nothing that would point to a polar differentiation, and in the 16-celled stage even this criterion is lost.

After the 4-celled stage is passed, the ovum of the opossum behaves no longer as a typical Eutherian, but as a marsupial ovum. In the former the blastomeres of the successive divisions cling together to form a solid mass or 'morula,' which is soon overgrown by a layer of cells, Rauber's layer or the trophoblast. The mass within is the 'inner cell mass' which gives rise to the embryo and its envelopes. The blastocyst is formed by the appearance of a cavity between the trophoblast and the inner cell mass at the lower pole of the egg.

In the marsupials the morula stage is absent. Already in the 2- and 4-celled opossum eggs the space between the blastomeres represents, potentially, the blastocyst cavity, for at the 16-celled stage, or even earlier, the blastocyst cavity is clearly indicated. As early as the 6-celled stage the blastomeres manifest a tendency to migrate to the zona pellucida and to apply themselves to the wall of the ovum (fig. 15, pl. 15). In 12- and 15-celled eggs the blastomeres are usually well flattened out at the periphery, as seen in figure 8, plate 3; figure 9, plate 13, and figure 16, plate 15. At the 16-celled stage it is exceptional to find rounded cells, and models of such eggs show the outer surface of the blastomeres molded against the curvature of the surrounding albumen (compare figs. 17 and 18, pl. 15).

It thus happens that the eliminated yolk comes to lie within the cavity of the blastocyst, for the blastomeres migrate to their

places against the wall of the ovum and here undergo further division and further flattening until they come into mutual contact and thus complete the blastocyst wall, leaving the yolk within the cavity. Figure 19, plate 15, is a section through an ovum of 26 cells; figure 20 through one of 28 cells. In both cases there are gaps in the wall of the blastocyst, indicating that this is not yet complete. The same is true of two eggs of 30 and 32 cells, respectively, in which the gaps are fewer in number (fig. 3, pl. 4). In figure 10, plate 13, and figure 1, plate 14, are shown sister ova of 32 and 34 cells, respectively; their walls are practically continuous and the blastocyst may be considered complete. Occasionally more advanced blastocysts still have gaps in their walls, as, for example, the one shown in figure 6, plate 6, which has 46 cells. We may say, however, that, on the average, the blastocyst wall is completed when the 32-celled stage is reached or soon thereafter. No polarity is evident in the egg, the cells being of uniform size and structure throughout. Not long after this the entoderm formation is initiated.

Hence, in the opossum the blastocyst is completed at a much earlier stage than in *Dasyurus*, where the blastomeres of the 16-celled egg are arranged in two superimposed rings at the equator of the egg. To form the blastocyst wall they must proliferate and migrate toward either pole, and the blastocyst is not completed until the gaps at the two poles are closed. In the opossum, on the contrary, to complete the blastocyst all that is necessary is the closing of the gaps between the cells which are early distributed more or less evenly at the periphery. The just completed blastocyst of *Dasyurus* contains more than three times the number of cells (90 to 130) than does the corresponding stage of the opossum, and it is three times as large.

At this stage in the opossum, neither the ovum nor its envelopes have increased perceptibly in size (pl. 12). The albumen layer lies over the ovum as thickly as before, again in striking contrast with the condition in *Dasyurus*, in which the albumen layer is completely resorbed when the egg has reached the 16-celled stage. The opossum blastocyst is completed about

thirty hours after the beginning of cleavage; in one case (No. 314) such eggs were found three and one-half days after copulation.

j. On the fate of the first two blastomeres

In the Eutherian ovum it seems probable that one of the first two blastomeres is destined to form the inner cell mass, the other the trophoblast, as was first pointed out by van Beneden ('75). If, then, Hill be correct in his interpretation of the embryonic area of marsupials as being homologous with the inner cell mass of Eutheria (a view in which I join), one might suppose that the 2-celled stages in the two groups of mammals are also homologous. But that this does not hold in the case

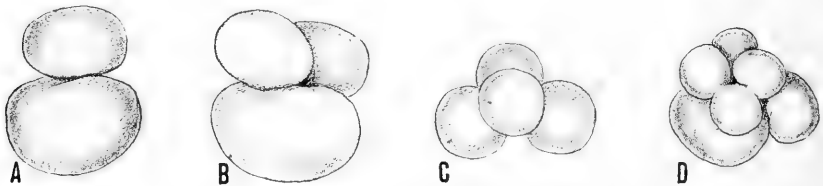


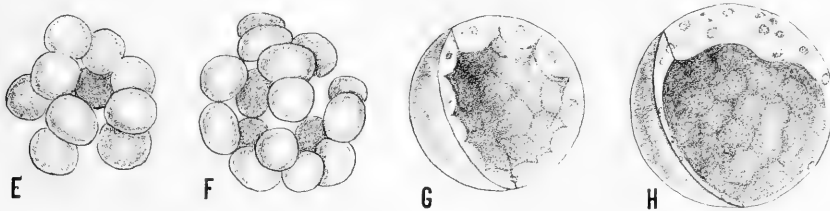
Fig. 5. To illustrate the probable fate of the two blastomeres of the 2-celled egg. Polarity is indicated in B, D, and E by the more rapid cell division at the upper pole. In F, a 16-celled egg, and G, one of 40 to 50 cells polar differ-

of *Dasyurus* seems clear from the scholarly work of Professor Hill. In *Dasyurus* the most reasonable interpretation of the facts is that the upper poles of the two blastomeres form the embryonic area and the lower poles the non-embryonic area. If this view be correct, then both blastomeres contribute equally to the embryo and to the trophoblast, or, in other words, the upper halves of the two first blastomeres of *Dasyurus* are together homodynamous with an entire blastomere of the Eutherian ovum, the lower halves homodynamous with the other blastomere. There would seem, then, to be a fundamental difference between the 2-celled Metatherian and the 2-celled Eutherian ovum.

The question arises: Does the opossum ovum follow, in its behavior, the egg of *Dasyurus*, with which the opossum is

phylogenetically more closely related, or does it follow that of the Eutherian ovum, to whose indeterminate type of cleavage it is strikingly and unexpectedly similar?

I have previously taken the latter position, namely, that the formative area very likely arises from one of the blastomeres, as in the Eutheria. If one follow a series of models of opossum eggs in successive stages, such as shown in text figure 5, A to H, one may visualize the formation of the blastocyst. We may safely assume that there are an upper and a lower pole in the eggs A to E, as evidenced by the difference in rate of division, aside from various other differences which may occur between the first two blastomeres (in size, amount of yolk extruded, rate of division). In the 12-celled egg there are eight smaller cells



entiation is lost, soon to be reestablished by the appearance of entoderm. It seems not unreasonable that the upper pole of H is the product of one of the two cells in A.

(2×4) and four larger cells (1×4), and it is evident that such an egg arose by one division from the 6-celled stage. Polarity is, therefore, indicated at least to this extent. The four undivided cells may next divide, establishing the 16-celled stage, in which polar differentiation is lost (F), not to be resumed again until the entoderm begins to proliferate at about the 50- to 60-celled stage (between G and H in the figure).

It is, therefore, impossible to bridge over the brief gap between the 16-celled stage, where polarity is lost, and the 50-celled stage, where it is resumed, and all that may be said is that it seems more reasonable to assume that all of the slowly dividing cells are of one kind and have one destiny and that all of the rapidly dividing cells are of another kind and have a different destiny. One has the choice between this view and the alterna-

tive, that a part of each type of cell goes into the formative and a part into the non-formative region.

Because of the short period in the opossum egg in which polar differences are lost, it is, therefore, impossible to demonstrate cell lineage in the cleavage of the opossum egg. But the same statement may be made with reference to the egg of *Dasyurus*, as pointed out by Hill himself, for, in the *Dasyurus* blastocyst, polar differentiation is lost during the long period of growth from 0.6 to 3.5 mm. Hill says ('11, p. 46): "It might therefore be supposed that the polarity, which is recognized in early blastocysts, and which is dependent on the pronounced differences existent between the cells of the upper and lower rings of the 16-celled stage, is of no fundamental importance, since it apparently becomes lost at an early period during the growth of the blastocyst. Such an assumption, however, would be very wide of the mark . . . and, indeed, in view of the facts set forth, is an altogether improbable one." There is not the slightest doubt that Professor Hill's view is the reasonable and the probable one. Upon the same grounds, the 2-celled opossum egg is not homologous with the 2-celled egg of *Dasyurus*, but rather with the 2-celled Eutherian egg.

Several abnormal opossum eggs are instructive in this connection, for they are indicative of polar differentiation in young blastocysts normally devoid of evidences of polarity. They are abortive attempts on the part of one-half of the egg in each case to form a blastocyst wall and they were found in litters of eggs made up for the most part of normal young blastocysts. Figure 3, plate 16, represents a section through an egg in which one-half of the blastocyst, consists of twenty cells, which have flattened normally, whereas the other half consists of eleven cells which are still rounded as in an earlier cleavage. Similarly, one-half of another egg (fig. 4, pl. 16) seemed to develop normally, the other half containing cells with fragmenting nuclei. In still another the normal half is beehive-shaped and surrounds two very large and three small cells (fig. 10, pl. 21). Egg No. 356 (2) has two large blastomeres at one pole of the blastocyst (fig. 14, pl. 22) and egg No. 88 (6) has a retarded blastomere enclosed within the blastocoele (fig. 13, pl. 22).

These cases perhaps indicate that the cells at one pole of the blastocyst are all of a distinct type, and it is not a far cry from eggs of 32 cells to the 2-celled stage, nor is it an unreasonable assumption, in view of the facts presented, to derive cells of each type from one of the two blastomeres.

THE FORMATION OF THE ENTODERM

a. General

In his classical work on *Dasyurus*, Hill has described an apparently new method of entoderm formation in mammals. His account is specific and definite, for the entoderm may be traced from certain unique cells which appear in the blastocyst wall when the egg has attained a diameter of about 4 mm. Within the embryonic area of such eggs a number of small ectodermal cells become modified, leave the blastocyst wall, and migrate to the inner surface to become the definitive entoderm. A similar process was independently discovered in the armadillo blastocyst and described in detail by Patterson ('13), who showed conclusively that also in this Eutherian mammal the entoderm forms not by delamination of cells on the surface of the inner cell mass, but by migration of the cells from the embryonic ectoderm of the monodermic vesicle.

Selenka, in his work on the opossum, naturally also speculated upon the method of entoderm formation in this species. His ideas were based upon one defective 8-celled egg and on two blastocysts of 42 and 68 cells, respectively. He believed that the lower half of the 8-celled egg consists of entodermal, the upper half of ectodermal cells. Each of his two youngest blastocysts has a large cell included within the blastocyst cavity, and one of his figures is almost identical with my specimen No. 314 (2), shown in figure 2, plate 5. This included cell, which he calls 'Urentodermzelle,' Selenka believed to be a migrant from the lips of the 'blastopore' at the 'entodermal pole' of the egg.

In my previous publication I reported upon 44 normal young unilaminar blastocysts, in 39 of which there occurred one or

more cells within the blastocyst cavity, as well as other enlarged and modified cells still within the wall (pls. 7 and 16; compare Hartman, '16, p. 36). I conjectured that the free cells might have arisen by accidental inclusion of a blastomere in about the 16-celled stage (compare fig. 17, pl. 15) or by proliferation from the large cells within the blastocyst wall, since frequently a number of cells would be united into a column projecting into the cavity (figs. 3 and 8, pl. 6). These cells appeared to come from various points in the blastocyst wall.

My next stage consisted of considerably advanced unilaminar blastocysts (compare figs. 1 to 4, pl. 18), in which I found entoderm in various stages of differentiation, including certain few cells that appeared to come out of the formative area of the blastocyst in precisely the same manner described for *Dasyurus* by Hill; and I figured cases in point.

With these two considerably separated stages before me, I concluded that the entoderm arose, as in *Dasyurus*, after the formative area had become well differentiated, and hence I considered the included cells of the young stages as of 'no morphological importance.'

Since publishing my report on these young blastocysts, I have been fortunate enough to collect an unbroken series of transitional stages between the just completed unilaminar blastocyst and the just completed bilaminar stage, and of especial interest are litters Nos. 344, 356, 194', and 349, of which I possess numerous preparations (pls. 8, 9, 16, 17). I also have more than five dozen additional young unilaminar eggs of the stage previously described, so that I now have before me 100 such preparations, besides a considerable number which I did not consider necessary to section. In these blastocysts I again find the persistent occurrence of the peculiar included cells such as previously described, which my new material now teaches are the *true entodermal mother cells of the opossum*. What I had previously described as entoderm formation marks the end and not the beginning of this process. The true entoderm formation begins in blastocysts containing 50 to 60 cells within the blastocyst wall; that is, these large modified cells in the blastocyst

wall, which proliferate after becoming free, or even in situ, constitute the first entoderm mother cells. This I am now able to show from the study of a closely graded series of stages, as abundantly illustrated by my drawings (pls. 16 to 18) as well as by photographs of preparations and of living eggs (pls. 6 to 9).

b. The youngest unilaminar blastocysts

It has been shown above that the blastocyst arises by the early migration of the blastomeres to the periphery of the ovum, where they flatten out against the zona pellucida or the albumen layer. By further division and spreading, the cells come into mutual contact, obliterating the spaces between them. The blastocyst is completed at the 32-celled stage or immediately thereafter. At first there is no evidence of polarity in the blastocyst, all of the cells being of the same structure and thickness throughout.

c. The first entoderm mother cells

At about the 50- or 60-cell stage, on the average, certain cells within the blastocyst wall undergo modification in situ. They become larger jutting out more or less into the blastocyst cavity. On their inner surface they may be rounded (*ENT*^A, figs. 6 and 7, pl. 16), or they may display an extended tip as if undergoing amoeboid movement (fig. 5, pl. 16). Some eggs show this tendency only to a slight degree in one or several cells; in others one or two cells will show more decided enlargement, projecting as much as two-thirds of the radius of the blastocyst into the cavity (fig. 4, pl. 7). These cells are the first entoderm mother cells in the opossum and can be traced in every gradation from earliest differentiation until they become detached from their place in the wall. Most of these cells are to be recognized only by their size and shape, since they have the same staining reactions as other unmodified cells and they contain apparently the same number of yolk granules. But if they remain some time in the wall, they elongate greatly and

take a much darker stain, as in figure 4, plate 7. This elongated type of cell is common in the collection. If the attachment of such a cell in the wall continues, it may give rise by cell division to columns of three, four, or more cells, as numerous examples serve to indicate (figs. 3 and 8, pl. 6, and figs. 20 and 21, pl. 16).

d. The detachment of entoderm mother cells

It more commonly happens, however, that the entoderm mother cells leave their place in the wall soon after attaining their maximum size, and their behavior at this time constitutes perhaps the most remarkable phenomenon in the entire development of the opossum egg. Their performance at this stage is little short of spectacular. Such partly or wholly detached cells are present in nearly every egg of litter No. 88, which covers this critical period in the formation of the entoderm by a series of more than two dozen preparations, and there are identical cells in numerous other excellent preparations from various litters. The cells, moreover, have such a characteristic appearance that I should term them the more typical entoderm mother cell of the opossum.

After a period of growth the entodermal cell rounds up on all sides. In this way its contour no longer conforms to the curvature of the ovum, and as a result, the contact with the adjoining cells is broken—the cell seems to roll out of its place, as it were, into the blastocyst cavity. But the gap thus formed does not long remain, for the vacant spaces are filled at once by a flowing in of the surrounding cells. This is clearly seen at A, figures 7 to 11, plate 16, which specimens were not selected originally with this point primarily in view, but they illustrate the phenomenon without exception. The entoderm mother cells, when they leave their place in the wall, do not, therefore, leave gaps that may be called 'blastopores' (Selenka), and such gaps as occur in earlier stages, with or without included free cells, are due to a different cause, as was shown above. Somewhat more advanced stages, moreover, still proliferate cells of the same type, as will appear below (pl. 17).

The newly formed entoderm mother cells are sometimes found in mitosis (fig. 10, pl. 16, and fig. 8, pl. 17); indeed, in egg No. 88 (11) six of the nine entoderm mother cells are in process of cell division, although most of them have not yet left the blastocyst wall (fig. 22, pl. 16).

It is thus apparent that the entoderm mother cells, found in variable numbers within the blastocyst cavity, arise from cells leaving the blastocyst wall and also as a result of their multiplication before, during, and after their migration into the cavity. The specimens figured here as well as numerous others afford ample evidence of these developmental processes.

The process in the opossum is essentially the same as obtains in *Dasyurus*, for, at a given stage in both forms, certain cells in the superficial unilaminar wall become modified and migrate into the interior of the vesicle. In the opossum we have an approach to the *Eutheria* in the early differentiation of the entoderm; hence we may consider the *Dasyurus* as exemplifying the more primitive, the opossum the more specialized condition.

e. Proliferation of entoderm confined to one pole

The small blastocysts of about 0.15 mm. in diameter referred to above cannot be oriented for sectioning, and hence the plane of the sections is entirely a matter of chance. It thus happens that the sections taken tangentially or obliquely through the ovum present, in some cases, very confusing pictures; for in such specimens the entodermal proliferation appears to take place promiscuously from various parts of the egg, and the polarity, which is very apparent in favorably cut series, is thus obscured. In the former the entodermal proliferation is palpably confined to one pole; to ascertain the arrangement in the latter it is necessary to make idealized reconstructions in the proper plane. This I did from series of camera-lucida drawings. Five such reconstructions are shown in figures 18 to 22, plate 16. In every case, without exception, the entoderm proliferation is confined to one pole, in some cases to exactly one-half of the blastocyst. We may, therefore, now speak of embryonic and

non-embryonic areas, for there is no longer any doubt as to their identity: the embryonic area is marked by the position of the entoderm mother cells and the polarity of the ovum is definitely reestablished.

A study of the young blastocysts just considered, as well as immediately succeeding stages, seems to show, moreover, that the first proliferation of entoderm takes place more actively on the margin of the future embryonic area, for one often finds them most numerous on opposite sides, as shown, for example, in figures 11, 18, and 22, plate 16, and figures 8, 10, and 11, plate 17.

The blastocyst now contains two types of cells: 1) those clearly entodermal in destiny, as just described, and 2) the peripheral or enveloping layer, which, of course, gives rise to all of the ectoderm, embryonic and trophoblastic. All of the cells at the lower pole are ectodermal, being trophoblastic. But the epithelial cells at the embryonic pole, since they will for some time still continue to proliferate entoderm mother cells, are potentially both ectodermal and entodermal and should better be called entectoderm until the entoderm is fully formed. They are, however, also potentially mesoderm, as my next paper will clearly show.

The ovum of the opossum has not grown much in volume since its discharge from the ovary, being still less than 0.15 mm. in diameter (pls. 12 and 13), which is in striking contrast with the blastocyst of *Dasyurus*, where, at the appearance of the first entoderm mother cells, the vesicle is nearly 4 mm. in diameter. In the opossum the period of growth follows the formation of the entoderm, but in *Dasyurus* this is preceded by a long period of growth. The process of entoderm formation in *Dasyurus* may, therefore, be studied from surface mounts of pieces easily cut from the blastocyst wall, as well as from serial sections; but the opossum egg at this stage may be studied in section only, for it is small, covered with a thick layer of albumen, and is densely packed with more or less opaque yolk. The vesicular structure can well be made out from in toto preparations, but for detailed study such preparations are worthless.

f. Time of appearance

From the foregoing it is apparent that size is as yet no criterion to the differentiation among the blastomeres. The number of cells seems, therefore, to be the best means of establishing the stage in question. With this in view, a careful count was made of the number of cells in thirty-three flawless series. By making camera-lucida drawings of each series sketching in the nuclei, superimposing the successive drawings, and eliminating duplicates, it is believed that the counts are quite accurate. This data is presented in table 5.

This table shows that there is a rough correlation between the number of cells and the extent of entoderm proliferation. Extreme variations occur, however, as, for example, in the sister eggs Nos. 298 (1) and 298 (3), which have four entodermal mother cells each, but the latter totals twice as many cells as the former (64 and 124, respectively). But it may be stated in general terms that entoderm proliferation usually begins when the blastocyst is made up of 50 or 60 cells.

It is thus apparent that in the early differentiation of entoderm the opossum again approaches more closely than does *Dasyurus* to the condition in the Eutheria. Thus in the absence of polar differentiation in the undivided egg (with due consideration to certain exceptions as the bat and armadillo), in the crossed arrangement of the blastomeres in the 4-celled egg; in the more or less indeterminate type of cleavage; in the early proliferation of entoderm—in all of these characters the opossum egg resembles that of the Eutheria. But in the absence of the morula stage and in the method of entoderm formation from definite entoderm mother cells arising from the unilaminar entectoderm the opossum closely resembles its relative *Dasyurus* as described by Hill. It is, of course, possible that *Dasyurus* represents the more typical development among the marsupials, as would appear also from Hill's description of some vesicles of *Macropus* and *Parameles*, in which the entoderm is laid down in vesicles less than 1 mm. in diameter, just as in the opossum.

TABLE 5
Cell counts in young opossum blastocysts

IDENTIFICATION NUMBER	NUMBER OF ENDODERMAL MOTHER CELLS	NUMBER OF CELLS IN WALL OF BLASTOCYST	TOTAL NUMBER OF CELLS	FIGURES AND PLATES WHERE ILLUSTRATED
314 (2)	2 (?)	28	30	2, V
314 (5)	0	32	32	4, VI
191 (2)	0	32	32	10, XIII
191 (5)	0	34	34	1, XVI
292 (4)	0	46	46	6, VI
88 (5)	0	50	50	
50 (3)	0	52	52	
88 (13)	0	62	62	
50 (7)	0	70	70	2, XVI
50 (5)	1	64	65	1, VII
83 (5)	1	52	53	12, XVI
50 (8)	2	61	63	5, XVI
88 (10)	2	50	52	
88 (23)	2	55	57	10, XVI
298 (1)	4	61	64	7 and 8, VI
298 (3)	4	20	124	20, XVI
356 (3)	—	—	100	13, XVI
88 (20)	5	60	65	
88 (7)	5	82	87	3, VI, 21, XVI
83 (1)	5	106	111	19, XVI
88 (1)	6	72	78	
88 (17)	6	97	103	2, VII; 18, XVI
298 (5)	8	118	126	6, XVI
50 (4)	9	59	68	3, VII
88 (11)	9	94	103	22, XVI
88 (3)	10	59	69	6, VII
88 (21)	10	60	70	8 and 9, XVI
88 (16)	10	72	82	4, VII; 11, XVI
88 (9)	11	95	106	7, XVI
344 (14)	19	174	193	16, XVI and 17
344 (11)	23	141	164	15, XVI
344 (7)	45	124	169	7, VIII
356 (4)	42	241	283	6 and 7, XVII
356 (5)	48	201	249	10 and 11, XVII

g. Included cells which may not be entodermal

It sometimes happens that a blastomere in early cleavage becomes displaced, fails to attain its proper position at the periphery and thus comes to be surrounded by its fellows. I have several 16-celled eggs with one such misplaced blastomere (fig. 17, pl. 15). Another egg, No. 314 (2), shown in figure 2, plate 5, has two included cells. One of these in the section figured is near a gap in the blastocyst wall, and it might be supposed that it had migrated from the unoccupied space. But this egg is made up of only 28 cells, a stage at which the blastocyst would hardly be expected to be completed. The cells were probably accidentally included at a somewhat earlier stage and are probably not typical entoderm mother cells. The included cell in ovum No. 83 (5) shown in figure 12, plate 16, has every characteristic of an entoderm mother cell except that it is unusually large. Some other included cells, as, for example, those in figures 10, plate 21, and 13, plate 22, are clearly undivided blastomeres of an early cleavage stage, as previously pointed out. The true entoderm mother cells are quite distinctive and are not readily mistaken for abnormal cells.

h. Further polar differentiation

After the proliferation of entoderm mother cells is well under way, the differences between the embryonic and the non-embryonic areas of the blastocyst become more and more pronounced. The former becomes marked by the large size of its cells as well as by the presence at that pole of entoderm mother cells; the non-embryonic portion becomes progressively more and more attenuated. These changes are readily understood from plates 16 and 17. The increase in size of the blastocyst is largely due to the spreading of the non-embryonic or trophoblastic ectoderm, and as a result the embryonic area comes to occupy a more and more restricted proportion of the surface of the vesicle, and this process continues until the formation of the bilaminar stage has been completed. The change in

proportionate number of embryonic and trophoblastic cells is apparent from the few examples given in table 6.

If a comparison be made between the facts shown in table 6 and the illustrations referred to therein, it is apparent that the increase in the number of cells and the differentiation proceed *pari passu*. In figure 15, plate 16—a longitudinal section through ovum No. 344 (11)—the formative area is roughly marked out by the position of the entodermal cells and the yolk and coagulum surrounding them; there is some thinning out of the trophoblastic cells. Ovum No. 344 (14) is slightly more advanced. It was cut tangentially to the formative area, to which nine of the twenty-two sections belong, the limits of this area being determined by the presence in the ninth section of

TABLE 6
Number of embryonic and trophoblastic cells number of cells

IDENTIFICATION NUMBER	TOTAL NUMBER	ENTODERMAL CELLS	EMBRYONIC ENTECTODERM	TROPHOBLASTIC ECTODERM	ILLUSTRATIONS, (FIGURES AND PLATES)
344 (11)	164	23	71	70	15, XVI
344 (14)	193	19	76	98	16, and 17, XVI
356 (4)	283	42	101	140	6 and 7, XVII
356 (5)	249	48	126	75	10 and 11, XVII

the last entodermal cells. The further differentiation between the two areas, as seen in litter No. 356, is quite apparent from a glance at plate 17.

In the eggs of litter No. 344, of which I have seven excellent preparations, the vesicular structure was quite apparent in the living state as well as after fixation, but it was not possible to show this in the photographs taken after staining them, since the albumen also absorbed considerable stain (fig. 2, pl. 6). Two eggs were, however, photographed alive in Ringer's solution and are shown in figures 5 and 6, plate 8. The former was taken in side view and shows the yolk and coagulum hanging in the vesicle like a bunch of grapes; in the sections of the egg taken longitudinally the relations were found to be as in life (fig. 7, pl. 8). The other eggs shown in figure 6 was photographed

with the embryonic area uppermost; the dark spot in the center is the rather opaque yolk mass.

In the eggs of litter No. 356 the polarity was always apparent in whatever medium they were placed, whether in Ringer's solution immediately on removal from the uterus or in alcohol after fixation; hence these eggs were readily oriented for sectioning. One of these eggs was photographed by transmitted light in salt solution and is shown in figure 1, plate 8. It is a perfect sphere, situated in the center of the egg as in younger stages (fig. 4, pl. 12; fig. 1, pl. 6). The embryonic area is an opaque mass at one pole and the trophoblastic area is a thin layer making up the rest of the vesicle. This egg is typical of all of this litter (fig. 1, pl. 6), all of which measure 0.17 to 0.20 mm. in diameter through the vesicle. Fixation and staining have not changed the relation of structures essentially and even the distortion due to imbedding is very slight (compare figs. 1 and 2, pl. 8; fig. 3, pl. 9, and fig. 1, pl. 6).

A somewhat transitional stage between Nos. 344 and 356 is furnished by litter No. 144 (figs. 1 to 3, pl. 17). These eggs were overfixed in Carnoy's fluid, but are instructive and corroborative of the trend of development described above.

In all of these litters (Nos. 144, 344, and 356) the entoderm mother cells are still being formed, as the figures in plates 16 and 17 amply show (ENT^A , ENT^1). The cells are of the same type as those previously encountered, namely, rounded and in process of leaving the periphery. Because of the greater density of the cytoplasm, these cells often take a deeper cytoplasmic stain than do the neighboring cells, from which they also become separated by a more definite cell membrane. Occasionally the entodermal cells are united into a column, as at ENT^2 , figure 5, plate 17, reminding one of such rows of cells in the younger stages (fig. 3, pl. 6).

In these eggs, too, the margin of the embryonic area seems to be the region of greatest proliferation. Thus figure 10, plate 17, represents a section near the margin of the area and shows a line of primitive entodermal cells; while figure 11, a

section nearer the middle of the series, shows entodermal cells only at the margin.

At the stage just described there is still a considerable quantity of yolk and coagulum in the egg. This is usually collected near the inner surface of the embryonic area as well as among or within the cells of the area, more rarely also in the trophoblastic cells. Occasionally the yolk is collected in a large spherule, as in egg No. 356 (11); this spherule measures 0.04 mm. in diameter and a portion of it, cut tangentially, is shown at Y, figure 4, plate 17.

i. The embryonic area superficial in position

The question may arise whether there appears at any time over the embryonic area a transitory layer that may at all be compared with Rauber's layer in the Eutherian egg. Professor Hill has homologized the embryonic area of the Eutherian egg with the inner cell mass and the non-embryonic area with Rauber's layer, and hence he uses the term 'trophoblastic' to designate the latter. According to this view, the embryonic cells lie upon the surface of the ovum from the beginning and are potentially ectoderm, entoderm, and mesoderm; in other words, the embryonic cells are never covered with trophoblastic cells. I believe this to be the true interpretation of the facts. Since my collection includes an unbroken series of critical stages on this point, if there were such a layer, it could not escape detection. Nowhere is there the slightest suggestion of a transitory layer of cells. Mitoses are always present in the superficial layer (pl. 17), disintegrating cells never. The very method of blastocyst formation, as described in these pages, precludes the probability of a trophoblastic cover over the embryonic area, which is differentiated very soon after the establishment of the blastocyst. For, if the upper half of the unilaminar opossum egg is not embryonic, it is trophoblastic and there can be no embryonic area; in which case we should be forced to derive the embryo from the trophoblast, a manifest absurdity. The preceding and the succeeding stages all show that in the blasto-

cysts last described, the superficial cells at the upper pole are ectodermal except a few which are destined to form entoderm mother cells.

j. The primitive entoderm

In the stages thus far described the entodermal cells are still round to polygonal and only occasionally does a cell flatten out upon the surface of the mass as at *ENT*³, figure 4, plate 17. We may call these cells primitive entodermal cells (*ENT*²) as distinguished, on the one hand, from the large entoderm mother cells from which they arose (*ENT*¹) and on the other, from the typical, flattened definitive entoderm into which they are about to develop. The primitive entoderm, through rapid cell division, becomes more or less crowded and shows a tendency to become two or three cells deep, as early as the stage represented by litter No. 356 (pl. 17).

k. Further growth of the blastocyst

When the blastocyst contains less than 200 cells, of which about 20 would be entodermal (litter No. 344, pl. 16), the embryonic and the trophoblastic areas each make up about one-half of the blastocyst wall. When the number approaches 300, including 40 or 50 entodermal cells (litter No. 356, pl. 17), the latter area has greatly extended so that the embryonic portion occupies a third or less of the blastocyst wall. The increase in size of the blastocyst is, therefore, to be attributed largely to the spreading and attenuation as well as a more rapid multiplication of trophoblastic cells (table 6).

The eggs of litter No. 194' are illustrative of the further development in the direction just indicated and follow close upon the eggs of litter No. 356. The vesicle has grown from about 0.23 mm. in diameter as the maximum for litter No. 356 to 0.34 mm. in litter No. 194', or about double the diameter of the ovum at cleavage. The blastocyst is still situated in the center of the egg, which has, however, not yet increased in

volume of shell membrane (text fig. 2; fig. 13, pl. 13; fig. 5, pl. 12).

The development of the trophoblastic ectoderm is seen to have continued in the direction indicated above, so that in this litter of eggs it now occupies about three-fourths of the circumference of the blastocyst. Little more need be said of this layer. It becomes progressively more attenuated until it may have the appearance of endothelium, and even at high magnifications appear as a sharp narrow line with here and there a swelling which marks the location of a nucleus (fig. 1, pl. 18). The region may come to occupy from four-fifths to five-sixths of the entire circumference of the blastocyst; and this again constitutes a point of contrast with the egg of *Dasyurus*, in which the formative area occupies, in section, from one-third to one-half of the blastocyst wall.

In general, the marsupial trophoblast does not differ markedly in structure from that of Eutherian vesicles, but more interesting and important changes take place in the embryonic area of the opossum blastocyst. For a short period, which includes the stage represented by litter No. 194' (figs. 13 to 15, pl. 17), these changes now appear to be chiefly of two kinds: 1) further proliferation of entoderm mother cells from the peripheral layer, and 2) multiplication of all types of cells.

The former process gives every evidence of having slowed down considerably since the preceding stage, the cells which can be identified as migrating inward from the superficial layer being of comparatively rare occurrence. Such cells are shown at *ENT*^A, figs. 14 and 15; they stand with their long axes at right angles to the surface of the area and project inward among the primitive entodermal cells now everywhere closely applied to the ectoderm. It is clear that entodermal proliferation from the superficial entectoderm is approaching the end.

As a result of the cell multiplication, the embryonic area has become crowded, so that in places it is three and occasionally four cells deep; and it may be stated parenthetically that this is the only stage before the formation of the mesoderm that the blastocyst wall is anywhere more than two cells deep, as the

sequel will show. At this stage there is no regular arrangement of entodermal cells into an epithelium, and, even in the superficial layer, regularity is only approximated (figs. 13 to 15, pl. 17). The cells which are not in contact with the albumen are as irregular in shape and size, at least in my specimens, as they are in arrangement; only the nuclei preserve a uniformity of size and structure.

The superficial cells for the most part are clearly embryonic ectoderm, and all of the nuclei seen below this layer are primitive entoderm. Most of them possess rounded nuclei, and only here and there in the sections is there any indication of cells which tend to flatten out into definitive entodermal cells (*ENT*², fig. 15). In this respect there has been little progress since the preceding stage.

Litter No. 194', was found four days after copulation or about two days after the beginning of cleavage.

A somewhat more advanced stage is represented by litter No. 349, one of which is shown photographed in the living state in figure 3, plate 8. It measures 0.32 mm. through the vesicle. Figure 4 is a section through the youngest egg of the litter and belongs to an earlier stage corresponding to litter No. 344. One of the two eggs like the one in figure 3 was sectioned, the other was accidentally broken and was used for study in toto. The two are in essential agreement. The formative area, shown as a distinct opacity in the living egg (fig. 3, pl. 8), is larger in area than in the litter just described; the trophoblastic area is thick-walled and less extended than would be expected at this stage. The embryonic area is crowded, the cells being three and four cells deep in some places. A large number of cells of all types—embryonic ectoderm, primitive and definitive entoderm—are in mitosis, chiefly in the spireme stage, as though a wave of cell division had spread over the entire area. Here and there an entoderm mother cell is still in process of formation. The definitive entoderm has begun to differentiate and to spread beyond the area (*ENT*). In surface view the embryonic area is approximately round and is sharply marked off from the surrounding trophoblastic ectoderm. This description shows

that the entoderm is present in a watch-crystal-shaped mass at one pole of the egg in vesicles of 0.30 to 0.35 mm. The mass is thicker in the middle, being even three to four cells deep. Only the outer superficial layer is ectodermal, the massed cells beneath being all entodermal.

The opossum blastocyst differs, then, both from the corresponding stage of *Dasyurus*, on the one hand, and of the higher mammals, on the other. In *Dasyurus* the entodermal cells flatten out and spread singly as they are formed and never pile up in a mass as in the opossum. There would seem to be in the opossum a nearer approach to the Eutherian ovum in its possession of a kind of 'inner cell mass' (fig. 15, pl. 17; fig. 1, pl. 18).

But there are fundamental differences. For in the Eutheria the entoderm seems to arise only from the cells on the inner surface of the inner cell mass, presumably from a single layer. The outer or superficial layer of the blastocyst is Rauber's layer; between the two is the embryonic ectoderm, a layer of cells variable in thickness and at first irregularly dispersed. Thus, if figure 1, plate 18, represented an Eutherian egg, the superficial layer would constitute Rauber's layer; beneath would be the irregularly disposed ectoderm (cells marked 'ENT²'), and only the innermost layer would be the entoderm. In the opossum there are only two layers: 1) embryonic ectoderm, a superficial layer, one cell deep, and 2) all the remainder which is entodermal. If there seem to be more than two layers, as in the figure just referred to, the outer layer is the ectoderm, the inner the differentiated entoderm, and between the two a mass of cells which are still undifferentiated or primitive entoderm which are yet to spread and form entoderm. The opossum is, therefore, fundamentally like *Dasyurus*; but the resemblance is obscured by the temporary massing of entodermal cells, the resulting picture superficially resembling an Eutherian vesicle with spreading inner cell mass.

1. The end of entoderm formation and the spreading of the entoderm

In my previous publication I described some vesicles from 0.3 to 0.5 mm. in diameter, of the type shown in figures 1 to 4,

plate 18, in which certain cells seemed to migrate from the embryonic ectoderm to take their place among the entodermal cells. I interpreted these cells as entoderm mother cells and presented a number of cases which closely parallel the process of entoderm formation in *Dasyurus* as described by Hill. Indeed, Hill states that in *Macropus* the primitive entodermal cells are already recognizable as cells situated internally in the blastocyst of 0.35 mm. and in *Parameles* in vesicles of about 1 mm., which would seem to correspond very well with the condition in the opossum. Certain cells drawn in figures 3 and 4, plate 18, might conceivably be proliferating entoderm. If this be true, then certainly these sporadic cases are the last stragglers in the stream of entodermal cells which arise from the entectoderm. The climax in the formation of entoderm in the opossum, however, occurs long before this, namely, in blastocysts between 0.15 and 0.30 mm. in diameter.

The differentiation of primitive entodermal cells into definitive entoderm takes place with rapidity soon after a diameter of 0.34 mm. is attained (litter No. 194'), so that in vesicles of about 0.50 mm. the entoderm has largely assumed its squamous structures and lies closely appressed against the simple unilaminar ectoderm. As soon as this differentiation is well under way, the entoderm at once migrates beyond its region of origin toward the opposite pole of the vesicle. These changes are readily observed in some typical examples furnished from litters Nos. 194', 349, 40, 43, 175', 339, 299', and 347.

In litter No. 194' as described above, the spreading of the entoderm has scarcely begun. In litter No. 349 (fig. 12, pl. 17) a few cells have flattened decidedly, while the majority are still in the condition of indifferent primitive entoderm. The tendency to spread is exhibited on the entire margin of the area. As soon as the entodermal cells have differentiated, they at once stain much darker, a characteristic which they maintain in sharp contrast to the ectoderm throughout the bilaminar stage; this is true without exception.

A somewhat later stage is represented by egg No. 43 (7), which is large, and has a greatly attenuated trophoblastic area,

and represents the normal condition at this stage. The section in figure 1, plate 18, is the tenth of thirty-five sections through the embryonic area; that is, lies to one side of the midline. The definitive entoderm would seem from this section to clothe the entire inner surface of the area, but a study of the series discloses the fact that the entoderm has differentiated only in spots, chiefly at the periphery of the area. That these changes take place chiefly in the periphery first would appear also from ovum No. 339 (3) shown in figures 6A and 6 and in figure 2, plate 6. At *ENT*² is a group of cells which run through ten sections; they are primitive entodermal cells not yet differentiated. So also in figure 8 (egg No. 175' (2)) the entoderm has already advanced considerably toward the equator, although there are still some undifferentiated cells near the middle of the area. Similar undifferentiated cells are also seen in other vesicles, as at *ENT*², figures 3 and 4.

Litter No. 352 consists of small blastocysts which fall into the stage under discussion. The group of eggs was photographed fresh in Ringer's solution by transmitted light (fig. 1, pl. 9). The vesicles with their more or less opaque embryonic areas are very evident. In the largest specimens the entoderm has entirely differentiated, except in one (fig. 14, pl. 13; fig. 8, pl. 21) in which a large blastomere has retarded the spreading of the cells (compare fig. 2, pl. 9, and fig. 2, pl. 18). In these eggs the entoderm has also advanced some distance toward the equator of the blastocyst.

m. Maximum attenuation of the blastocyst wall

The trophoblastic ectoderm, it was seen above, begins its thinning and spreading process soon after the proliferation of entoderm begins (in 0.15-mm. ova) and reaches its maximum when the formation of new entodermal cells from entectoderm ceases, and the entoderm begins to line the lower hemisphere (0.50-mm. blastocysts). The spreading and attenuation, however, affect the embryonic as well as the trophoblastic area and takes place rapidly while the entoderm is migrating to the

opposite pole. In all of the eggs of litters 175' and 347 (pl. 18) these facts are clearly shown. Blastocyst No. 175' (9) is an extreme case in point (figs. 7 and 7A, pl. 19). In succeeding stages the embryonic area thickens progressively but slowly, until it reaches its maximum in blastocysts 1 to 1.5 mm. in diameter, after which it remains more or less constant until the embryo begins to differentiate.

n. Cause of spreading of the entoderm

In the spreading of the entoderm the chief factor is the active migration of the entodermal cells. The passive spreading, due to the enlargement of the vesicle, as had been suggested in the case of other mammalian vesicles, is, in the opossum, a negligible factor. An inspection of plate 18 will make this clear, for the vesicles are about as large when the entoderm begins to spread (fig. 1) as when it has reached the opposite pole (fig. 5). In fact, comparison of eggs of the same litter (figs. 5 and 7) show that the size of the vesicle bears no relation to the extent of the entoderm. My observations on numerous eggs at this stage (Nos. 347 and 299') go to show that, when once begun, the spreading of the entoderm proceeds rapidly. I have not been able to demonstrate amoeboid movements in the cells, but processes sometimes occur on entodermal cells at about this stage (fig. 2, pl. 19).

o. Changing position of the vesicle in the egg

In most of the eggs which mark the early stages in the spreading of the entoderm, the vesicle still occupies practically the center of the eggs as in previous stages (text figs. 1 and 2). In litter No. 352 (fig. 1, pl. 9) the tendency of the vesicle to approach the shell membrane is already manifest to some extent; also in litter No. 175' (fig. 7, pl. 19). In all later stages the vesicle occupies an eccentric position and in most cases it is in immediate contact with the shell membrane. This contact is established, therefore, for the first time about the beginning of the bilaminar stage, or about four days after the beginning of

cleavage. This delay is in contrast, again, with the egg of *Dasyurus*, in which the blastomeres in the 16-celled stage have already established contact with the shell membrane on all sides, the albumen of the egg, always limited in thickness, having entirely disappeared. The formative area almost always reaches the shell membrane first, the exception being very rare. The albumen, therefore, becomes concentrated at one pole, gradually decreasing in amount with the growth of the blastocyst. Henceforth the stage of advancement of the blastocyst may be gauged by the amount of albumen which appears as a crescent in the egg when viewed from the side or when seen in a longitudinal section (eggs No. 299', in figs. 1 and 2, pl. 6; fig. 5, pl. 10, etc.). That this eccentric position is not an artifact due to fixation or other causes, is shown by the fact that in the living egg the blastocysts are situated in exactly the same position as after fixation, as the photographs (fig. 4, pl. 1; figs. 4 to 6, pl. 9) show.

p. Some abnormal eggs

Since future workers on the opossum are likely to encounter abnormal material, it is not amiss to describe several abnormal eggs of about the stage just described.

In the group of eggs in figure 2, plate 6, a number of such abnormal specimens are shown: Nos. 294 (1), 294 (2) and 294 (3) and 339 (4). The last mentioned is the least abnormal of all. In the living state the vesicle was spherical and remained so throughout the process of imbedding (figs. 5 and 5A, pl. 19). A similar egg is shown in figure 15, plate 13, and the embryonic area of a third in figure 6, plate 19. These eggs, all from one litter, are in close agreement and the relation of ectoderm and entoderm is as in normal eggs of this stage (compare pl. 18). But the wall of the vesicle consists of unduly inflated cells with very diffuse cytoplasm and often large nuclei. Egg No. 339 (3) is exceptional in this litter, for its normal appearance; it doubtless represents the normal stage to which the others should have attained (fig. 6, pl. 9; fig. 2, pl. 6; figs. 6 and 6A, pl. 18).

The other more abnormal eggs referred to above show even at low magnification evidences of abnormality (fig. 2, pl. 6). The vesicles are not plump and rounded, but more or less shriveled and are surrounded by a large 'perivitelline space.' That the abnormalities are not due to the method of fixation is shown by the appearance of the living eggs, of litter No. 294 reproduced in figure 1, plate 11. In sections made of these eggs the walls are composed of cells swollen to enormous volume and are extreme cases of the condition shown in fig. 5A, pl. 19. Many cells are in mitosis, with the chromosomes strewn about pell-mell throughout the cell. Similar eggs are also met in normal litters (fig. 4, pl. 9). The 'pear-shaped vesicle' described by Selenka and figured in his *Tafel XVIII*, Fig. 1 u. 2, was doubtless an egg of the type just described.

PART IV. THE BILAMINAR BLASTOCYST

GENERAL DESCRIPTION

a. Material

The various stages in the bilaminar blastocyst of the opossum are represented in my collection by an unbroken series separated from one another by minutes rather than hours of development. Two hundred and thirty-five normal eggs were secured from thirty-two litters of twenty-five different animals; hence it may be assumed that the following description gives in detail the normal opossum egg during these stages. One hundred and fifty eggs were sectioned or were dissected for study of surface views; and the former include many that were carried through the imbedding and sectioning process without collapse and with the minimum of shrinkage.

b. The living eggs

The general trend of development during this period may be followed by reference to the photographs of living eggs presented in plates 1, 2, 9, and 11 of this paper.

All of the eggs still lie free in the lumen of the uterus (fig. 10, pl. 1; fig. 8, pl. 2) and are distributed as in the preceding stages, often grouped near the os uteri; hence one should not speak of the 'implantation' of the eggs even at the 2-mm. stage. The shell membrane has attained considerable thickness (Hartman, '16) and throughout the stage in question maintains the shape of a perfect sphere.

Before the entodermal spreading is well under way the blastocyst occupies approximately the center of the egg (fig. 1, pl. 9). Before the entoderm has reached the opposite pole of the blastocyst the embryonic area has almost or quite come into contact with the shell membrane (compare figs. 3 and 4, pl. 19), giving the blastocyst a decidedly eccentric position. It now fills one-half or less of the egg and has the shape of a bi-convex lens (fig. 5, pl. 10). This migration of the blastocyst may be due to the increased metabolism of the more voluminous cells of the embryonic area, as a result of which the albumen is here more rapidly digested and absorbed. This position is maintained in the subsequent stages (compare eggs No. 299', fig. 1, pl. 6).

The size of the entire egg containing the youngest bilaminar blastocysts with just closed entodermal sac is very little greater than the youngest uterine eggs, although the albumen has become denser and the vesicle wall has become considerably differentiated. Thus, for example, the diameter of the eggs in figures 4 to 6, plate 9 (all bilaminar blastocysts), is only a little greater than that of eggs in cleavage stages (figs. 1, 3, and 5, pl. 1). Again, the two litters shown in figures 3 and 4, plate 1, exhibit an evident, but not striking growth in volume, although they have developed from the 4-celled stage in the former to young bilaminar blastocysts in the latter in a period of four days, or 40 per cent of the entire period of gestation!

In all of the young bilaminar blastocysts the embryonic area is plainly outlined and distinctly marked off at the junctional line from the trophoblastic region. This differentiation increases with the growth and development of the egg.

From the beginning, the bilaminar stage is essentially the period of growth; this period thus follows the formation of the

entoderm, whereas in the *Dasyurus* it precedes as well as follows the process. At first the blastocyst grows faster than the shell membrane, for gradually the albumen disappears before the advancing trophoblastic area. This growth would seem, therefore, to affect the trophoblastic more than the embryonic area, which latter lies in contact with the shell membrane from the earliest bilaminar stage. The embryonic area, however, easily keeps pace with or even exceeds the rate of growth of the entire vesicle; for in the 1-mm. eggs it is proportionally larger than in certain younger stages. It would seem, then, that, despite the absence of albumen, the area receives sufficient nutriment for vigorous growth or is indeed better supplied by virtue of its superficial position in the egg, with the secretion of the uterine glands.

Eggs about 0.8 mm. in diameter, as illustrated by litter No. 306', removed four and one-half days after the beginning of cleavage, still contain considerable albumen which is readily visible at all positions of the eggs and may be seen on the photographs of the eggs (fig. 7, pl. 10; fig. 17, pl. 13; fig. 1, pl. 21). When the diameter of 1 mm. is reached, the quantity of albumen has been considerably reduced and is mostly confined to the trophoblastic region below the equator of the egg (fig. 2, pl. 21). In living specimens of such eggs the albumen is visible as a narrow crescent, only when viewed from the side, and is not visible in photographs of living eggs (fig. 5, pl. 2). The reduction of albumen continues, and when the diameter of the egg approaches 2 mm. and the mesodermal proliferation is about to begin there is only a thin film of albumen left (fig. 6, pl. 2; fig. 20, pl. 13). The amount of albumen at any stage is, of course, variable. It may still occur in small amounts in early primitive-streak stages (fig. 4, pl. 2; fig. 22, pl. 13).

The opossum blastocyst, therefore, begins as a perfect sphere at about the 32-celled stage. It maintains this shape until the definitive entoderm begins to spread, when the blastocyst assumes a biconvex form, flattened in the direction of the egg axis, and lies with the formative area against the shell membrane. The spherical form is again attained when the trophoblastic area

has reached the shell membrane, which occurs almost completely when the egg is 1 mm. in diameter, more perfectly at a diameter of 1.5 to 2 mm. It then maintains the spherical form until, through crowding of large litters in the pregnant uterus, the vesicles are somewhat misshapen through mutual pressure.

The embryonic area of the larger blastocysts, due to its protoplasmic differentiation, now stands out clearer, so that it is plainly visible in living eggs. It is recognizable in photographs of living eggs, but much more clearly in photographs of eggs immersed for a few minutes in the fixing fluid (fig. 2, pl. 11).

The size of the embryonic area varies greatly, even in proportion to the total surface area of vesicles in the same litter. In general its diameter occupies between one-fifth and one-fourth of the circumference of the egg, occasionally a little less than one-fifth, sometimes nearly one-third. But it never reaches the equator as in *Dasyurus*. An average 1 mm. blastocyst is shown in figure 9, plate 21. Various measurements are given under the legends of the eggs illustrated.

Aside from these details, little may be learned from a study of the living egg, and we must turn to preparations for more intimate details of structure. The progress of development will be followed by describing first the youngest stage, then the 1 mm. blastocyst, and lastly the blastocyst just preceding the proliferation of mesoderm.

THE JUST COMPLETED BILAMINAR BLASTOCYST

The bilaminar stage may be said to begin when the entoderm has migrated to the trophoblastic pole of the egg opposite its point of origin, thus forming a closed sac within the ectoderm. This stage was attained by most of the eggs in litter No. 299' about four days after the beginning of cleavage. The vesicle occupies about one-half of the egg contents.

a. The embryonic ectoderm

In the youngest bilaminar blastocysts the embryonic area is approximately circular in shape and clearly visible, but not as

clear-cut nor as definitely and neatly circular as in later stages. In surface view of preparations the junctional line between the two areas can always be made out (figs. 11 and 12, p. 19); but in sections some difficulty is experienced in trying to determine with exactness the marginal cells of either area, for the cells of the embryonic area have not yet assumed that density of protoplasm characteristic of later stages. The embryonic ectoderm is at first comparatively thin, consisting of a single layer of somewhat flattened cells as in figures 5 to 7, plate 18, and 3 and 8 to 10, plate 19. The area gradually thickens and the cells become cubical in section (fig. 4, pl. 10, fig. 4, pl. 19). In the younger eggs the nuclei are, therefore, further apart, but, as they multiply, they become more and more crowded until they are almost or quite in contact (compare fig. 3B, pl. 20, and fig. 12, pl. 19). In surface views the area is studded with mitotic figures (fig. 3B, pl. 20).

The surface views presented in figures 3B, plate 20, and 12, plate 19, of which the latter is the more advanced, show that the junctional line between the embryonic and the trophoblastic areas is quite definite, sometimes being marked by a continuous sharp line around the entire area. Figure 3B, plate 20, is especially instructive in this connection, since it represents in surface view ($\times 500$) a portion of the same area shown in figure 3A in section ($\times 200$); it is the portion removed before imbedding from point A, figure 3. There is, neither in the section nor in the surface view, any great difference of tone between the two areas, but the junctional line (XX) or margin of the embryonic area may easily be located where the ectodermal nuclei become less crowded. The junctional line is always more clearly defined in specimens fixed with a fluid that will bring out the cell membranes, as in figure 12, plate 19. It is seen that the line is formed of the cell membranes of contiguous cells bordering the areas. It is a perfectly definite structure: the marginal cells of the two areas do not intermingle nor do transitional cells occur between the two types.

b. The trophoblastic area

The cells of this area are very attenuated in the late unilaminar stage, but as they multiply they also thicken and increase their volume. The thickening is often most pronounced at the vegetative pole where numerous mitoses may occur (figs. 5 and 7, pl. 18). This is a matter of some interest, for in larger blastocysts this region may continue to be more thickened than the remainder of the trophoblastic area; or certain 'blisters' may occur there, such as will be described under the 1 mm. blastocyst (0, figs. 2 and 3, pl. 21).

In all stages the cytoplasm of the trophoblastic cells is very diffuse and loosely reticular, but in the early bilaminar stage especially it tends to break down into strands and reticulum, leaving the cell membrane collapsed and wrinkled. Only around the nuclei is there a denser mass of well-fixed cytoplasm and the nuclei themselves maintain their form and structure as perfectly as in the embryonic area (fig. 2, pl. 20). Normally, the trophoblastic layer follows the curvature of the albumen layer to which it remains closely applied, as is apparent from a study of the living eggs and photographs of them and as the best preparations show (fig. 4, pl. 1; fig. 17, pl. 13). Frequently, however, the vesicle collapses more or less in this area, leaving as an artifact a space between the vesicle and the albumen (fig. 4, pl. 10; *ART*, fig. 1, pl. 19). At this stage the albumen seems to be most dense nearest the shell membrane and often very loosely layered near the vesicle, which would account for the more frequent breaking down of the albumen at this point in the specimens.

c. The entoderm

Soon after completely lining the blastocyst wall the entoderm is everywhere the same, passing over the junctional line without change. This condition remains so throughout the bilaminar stage, except for certain modified cells to be mentioned in connection with older blastocysts. At first the entoderm is extremely delicate so that it appears in section to be discon-

tinuous, because in the fixing fluid portions of the cells break down. That this is the correct explanation is seen from surface views of good preparations, as in figure 11, plate 19, in which only the entodermal cells are shaded. They are seen to be connected by fibrous strands, the coagulated portions of the delicate cells. The entoderm may, therefore, be considered practically continuous. In most surface mounts the entoderm appears like a mottled surface, for the cells are thick in the middle, hence darker, and shade off almost into nothingness toward their edges (fig. 2, pl. 19).

The entoderm invariably stains darker than the ectoderm. It is interesting to note that an entodermal cell in mitosis is darker, often very decidedly so, than its fellows in the resting stage (fig. 2, pl. 19); while on the other hand an ectodermal cell is usually much lighter when in mitosis (fig. 12, pl. 19), so that in some surface views dividing cells look like holes in the wall. Yolk granules abound among the cells of the embryonic area, both ectodermal and entodermal, and occur occasionally also in the trophoblastic region.

A somewhat older egg (figs. 4 and 4A, pl. 20) is presented because of the degenerating cells included within the cavity. Such cellular remnants have been noted in apparently normal vesicles of *Eutheria* (Hartman, '16, pp. 46 and 47). In this egg, too, the albumen is definitely arranged in three layers of varying density, a condition noted also in a few other specimens.

THE 1 MM. BLASTOCYST

a. General description

The typical 1-mm. blastocysts contained in litter No. 343 (fig. 6, pl. 2) were removed seven and a half days after copulation. It has already been pointed out that only a small amount of albumen still remains in these eggs and the vesicle has become very nearly a perfect sphere. The embryonic area is now more sharply marked off from the surrounding trophoblast and lies like a cap at one pole of the egg (fig. 9, pl. 21). It is, in fact, occasionally in alcoholic specimens raised in relief

above the surface of the ovum like a blister, a condition probably due to its greater density and resistance to shrinkage as compared with the trophoblastic area.

The growing contrast between the two regions of the egg, which is now as clear-cut in sections as in whole mounts, is due to the increasing difference in the structure, as well as to the number of the formative cells. These are taller, much more crowded, and contain a denser and more granular cytoplasm, and this contrast in the types of cells is a constant character, no matter what the fixation, and the differences that exist among the specimens are those of degree only. These points are evident from an inspection of figures 1A, 2A, and 4 to 7, plate 21, which were drawn as nearly as possible in imitation of the tone of the specimens.

While there is great variability in the thickness of the embryonic areas in 1-mm. blastocysts, it is true that in most cases the area has become considerably thickened as the vesicle has grown in volume and as the area has increased in diameter (pl. 21). The cells have become mostly tall cubical to columnar and in the embryonic area are now nearly or quite as much crowded together as in older stages (compare fig. 12, pl. 19, and fig. 12A, pl. 22).

The embryonic ectoderm is arranged strictly in a single layer, never stratified or pseudostratified. The nuclei are practically on a level throughout, and this is one of the points of contrast with the blastoderm of other mammals. Mitotic spindles usually stand with their axes parallel to the surface of the egg (fig. 7, pl. 21). Frequently the cell that is in mitosis juts out above the level of the ectodermal layer (fig. 5, pl. 21), as in the blastocysts of the rabbit and other mammals, and such cells almost always stain less deeply, a fact that applies both to sections and to surface views.

The trophoblastic area has also developed more mass and thickness, proportionally quite as much as the embryonic area (figs. 6 and 7A, pl. 21). Typically it is about 8 to 10 μ in thickness. It is usually uniform in structure at all points and fits closely to the albumen (fig. 6), except when artificially separated

from it in fixation (fig. 7A). In the 1-mm. blastocyst it will endure fixation better than in younger stages. In all cases, in contrast with the uniform granulation of the embryonic area, the trophoblastic cells are reticulated and often possess coarse meshes or appear highly vacuolated. In extreme cases, especially when fixed in aceto-osmic-bichromate, the trophoblastic area may be greatly swollen; but this may also sometimes happen even in so reliable a fluid as Bouin's, as in figure 4, plate 22. The trophoblastic area is thus much more affected by fixation than the embryonic area.

While, as a rule, the trophoblastic area is rather uniform in thickness throughout its extent, there are frequent exceptions which deserve special mention. The area may gradually thicken toward the lower pole (fig. 4, pl. 22), or there may be a thick mass of cells jutting out into the albumen, and even touching the shell membrane at that point. In such cases the entoderm is continuous over the mass. In still other cases the ectoderm at the extreme lower pole is depressed outward into a pocket which may also come into contact with the shell membrane (*O*, figs. 2 and 3, pl. 21). The entoderm bridges over this cavity in a continuous layer and does not follow the ectoderm into the pocket. In the whole egg the pocket is quite evident and looks like a blister on the vesicle. These structures can scarcely have any special significance, since they are not of constant occurrence, nor are they situated at a point of special future importance.

As in both younger and older stages, the entoderm is a continuous layer lining the entire cavity of the blastocyst. It consists of a very attenuated layer of large squamous cells quite typical of the corresponding stage of all mammals. The cytoplasm is mostly gathered near the center of the cell, where the nucleus lies. In surface views the entodermal nuclei usually appear larger than the ectodermal and the chromatin granules in them are more evenly distributed. They can be recognized by this difference as well as by the depth of focus required to see them. The entoderm always has a stronger staining reaction than the ectoderm; I find no exception to this rule.

b. The bilaminar blastocyst according to Selenka

In his 'Studien' ('87) Selenka briefly describes two opossum blastocysts of about 1.1 mm. in diameter. The lithographs presented by him are idealized drawings, reconstructed from his sections, which I judge to have been considerably shrunk by the treatment to which they were subjected. The illustrations give the correct relation of the structures except for the diffuse junctional line, although Selenka is in error as to the homology of the 'Granulosa membran,' as he terms the shell membrane.

c. The 1-mm. blastocyst according to Minot

In 1911 the late Professor Minot published a description of six 1-mm. blastocysts of the opossum, of which two were fixed in Flemming's fluid and four in Zenker's and of the latter, two were fixed in situ with the uterus.

He gives an adequate description of the embryonic area of this stage, as did Selenka in 1887. Of especial interest, however, is Minot's description and interpretation of certain cells in the trophoblastic area. In a vesicle which he dissected and mounted flat on a slide he found numerous large light areas apparent as 'minute round holes' when viewed with a hand lens. He interpreted these areas as gaps in the ectoderm filled with entodermal cells which thus reach the surface at these points. Corroboration was found in the study of the serial sections. The author furthermore draws a comparison between these large lightly staining entodermal cells, which rise to the surface in the trophoblastic region of the opossum egg, and the small darkly staining entoderm mother cells which appear in the embryonic ectoderm of the *Dasyurus* blastocyst.

While the preparation of the present paper was in progress I had the privilege of studying Professor Minot's specimens at the Harvard Medical School. As I expected, the serial sections of eggs fixed and sectioned in toto with the uterus are badly shrunk and filled with coagulum in a manner which never occurs in eggs removed from the uterus and treated separately.

The specimens are unique, too, in that the entoderm is somewhat lighter in stain than the ectoderm, as described by Minot.

The surface mount is nicely fixed and is, histologically, an excellent preparation. The light areas are as described by Minot, and they are even more striking in the specimen than in his figure 2B. It is my judgment, however, that the vesicle in question is not entirely normal, for the reason that among all of my numerous specimens, I have never encountered any possessing such large light spaces. It is true that normally small lightly staining areas occur in almost all opossum vesicles of about this stage; and they usually mark the presence of cells in mitosis (figs. 12 and 12A, pl. 22), or cells that have just divided or are preparing to divide, and they are especially prominent in specimens fixed in bichromate mixtures. But they never attain such size as in Doctor Minot's unusual specimen. I have no explanation to offer of the phenomenon; I saw no evidence of degeneration of cells at those points.

Again, among all of my specimens I have looked in vain for entodermal cells coming to the surface in bilaminar blastocysts, either in surface views or sections; and I am certain that normally this does not occur. I have convinced myself, however, that also in the Harvard specimen the entoderm is nowhere at the surface and that Doctor Minot was in error in his interpretation. In the first place, by careful focusing with the oil-immersion lens the (ectodermal) nucleus within the light area is in several instance seen to be superimposed over an entodermal nucleus. Furthermore, if one plot the entodermal nuclei of the embryonic area, it is seen that they are uniformly and continuously distributed, entirely without reference to the above-mentioned light areas. These areas are without doubt ectodermal and not entodermal. Hence Minot's comparison between the supposedly superficial entodermal cells in the trophoblastic area of the opossum with the entoderm mother cells of the unilaminar blastocyst of *Dasyurus* is a futile one.

The entoderm, therefore, never comes to the surface in the bilaminar stage of the opossum egg. Entodermal cells in *Dasyurus* and in the opossum, and doubtless in all marsupials,

occupy the superficial position only as undifferentiated entoderm mother cells from which all of the entoderm is destined to be formed. The formative area is, from the beginning, potentially ectoderm, entoderm, and mesoderm, giving rise first to the entoderm and later in quite a similar manner to the mesoderm, the residue becoming definitive ectoderm. The trophoblastic area consists of a single layer, the ectoderm, until lined with the entoderm arising from the embryonic area.

THE LATE BILAMINAR BLASTOCYST

a. General description

Passing now to the later stages, we note that superficially the blastocyst appears to have changed but little, except in size (compare figs. 3, 5, and 6, pl. 2). The embryonic area remains prominent at the upper pole and less and less albumen remains at the lower pole. Important changes in the blastocyst wall are, however, to be discovered from a study of the sections.

In blastocysts of 1.2 to 1.5 mm. the ectoderm, both embryonic and trophoblastic, has attained its maximum thickness for the bilaminar stages. Two eggs shown in plate 22 illustrate this point. Egg No. 353 (4), shown in figures 3, 3A, 3B, 3C measured 1.22 mm. in alcohol. The embryonic area consists of tall cells, for the most part of the columnar type; the trophoblastic area is the same as in smaller blastocysts above described. Practically the same holds true for egg No. 360 (4) (fig. 6), which measured about 1.3 mm. in diameter (compare stereogram fig. 8, pl. 10). The wall of a somewhat smaller egg, No. 347' (1), 1.1 mm. in diameter in alcohol, has a somewhat thinner embryonic area (fig. 5, pl. 22). There is, however, considerable variation in this respect, even within the same litter of eggs of equal size.

In all of the larger blastocysts the entoderm can be followed as a continuous layer completely lining the vesicle. Except where pulled away in the preparations, the entoderm fits closely against the ectoderm and is always distinctly recognizable at all points (fig. 6, pl. 10).

b: The central light field in the embryonic area

When the diameter of the egg approaches 1.8 mm., certain changes of importance have taken place, for in such eggs the first proliferation of mesoderm is usually observed. The eggs of litters Nos. 189' and 353' are of this size; in the last litter mesodermal cells occur and the primitive streak is faintly indicated (*M*, fig. 21, pl. 13); but the other litter lacks a few minutes of development to have reached this stage.

The premesodermal changes in the blastocyst are best illustrated by a typical and favorably sectioned example, namely, egg No. 193' (2), measuring about 1.4 mm. in alcohol. This is one of the two eggs shown in figures 1 and 2, plate 10. In surface view there is within the embryonic area, a large light field, more plainly visible by transmitted light. Such areas have been described for other mammalian vesicles of a corresponding stage. They are usually somewhat eccentric, sometimes very considerably nearer one side than the other. I am convinced that the point where the light field comes nearest the margin of the area marks the posterior portion of the embryonic area, and I have therefore, in figure 9, plate 22, oriented the surface view of such an egg with the posterior end down. At a slightly later stage, perhaps an hour later, the primitive streak would have appeared as a faint tongue-shaped clouding projecting upward from the lower margin of the formative area into the light field in question, as in the eggs of litter No. 353', to be described in the next number of these studies.

The light field is due entirely to a thinning of the embryonic ectoderm (*T*, fig. 9A, pl. 22), a condition already seen in small eggs in figure 6, where at *T* the area is palpably thinner than at either end of the section. At *T*, figures 4A and 7, the sections also pass favorably to show this central thinner field. In this region, too, the nuclei are usually farther separated, whereas at the margins they are so crowded as to form a continuous chain like a string of beads, although not quite so uniformly arranged.

c. Modified entodermal cells

In many of these larger eggs the entoderm undergoes slight differentiation at one point. Over the junctional line which marks the border of the embryonic area there is often a group of entodermal cells which attract attention by virtue of their number, the roundness of their nuclei and the volume of the cytoplasm (*ENT*, fig. 9A). They are sometimes found in eggs of 1 mm. (*ENT*, fig. 5, pl. 21), more often in larger blastocysts (*ENT*, figs. 4A, 6 and 7, pl. 22). Similar entodermal cells have been described by Van Beneden for the bilaminar blastocyst of the rabbit. He states that they mark the anterior end of the area and that the future primitive streak appears at the opposite side. In the preparations made from litter No. 353', in which there occurs the first anlage of the primitive streak, these cells appear in the region where the mesodermal cells are found, hence not in the anterior, but in the posterior portion of the embryonic area. I shall treat this subject further at a later date.

d. The ectoderm of late bilaminar blastocyst

As was stated in the preceding section, the entoderm attains its maximum thickness in vesicles of about 1.3 mm. diameter (figs. 3A and 3B, pl. 22). Larger vesicles may have thinner formative areas or they may remain about the same, although they are apparently more slender because of their length in sections. In some of the eggs, especially in litters Nos. 193' and 343', the trophoblastic areas are as greatly attenuated as in the 0.8-mm. stage (figs. 1 and 9A, pl. 22). Sometimes the trophoblastic area becomes gradually thicker towards the lower pole (fig. 4, pl. 22), or there may be ectodermal pockets or 'blisters' at this point, as described above in connection with the 1-mm. stage.

As seen in surface view, the distribution of cells is practically the same as in the 1-mm. blastocysts (figs. 12A and 12B, pl. 22). All of the cells of the embryonic ectoderm are crowded closely together and are darker than the trophoblastic cells because

they are thicker and uniformly granular, but the cell boundaries are not as apparent as those of the large flat trophoblastic cells. The nuclei of the latter are flatter, but of uniform roundness, unchanged by mutual pressure, and possess fewer chromatin granules and larger light spaces than the embryonic nuclei, otherwise the nuclei of the two areas are very much alike. The entodermal nuclei, as a rule, appear larger in surface view than those of the ectoderm and they possess a more uniform granulation.

It should be noted that the embryonic ectoderm is still a single layer of cells with the nuclei mostly at nearly the same level. In the corresponding stage of other mammals the embryonic area is considerably thickened as in a pseudostratified epithelium (rat, bat). This simple arrangement has the decided advantage for the observer in that the very first mesodermal nuclei which drop down out of the ectoderm may be located instantly and with certainty.

e. Yolk spherules in ectoderm and entoderm

In many surface views of bilaminar blastocysts round dark objects, usually as large as a nucleus or smaller, frequently meet the eye. Sometimes these bodies stain like the cytoplasm, or they may be much darker in preparations fixed in osmic acid. They are found in both ectoderm and entoderm (figs. 1A and 2A, pl. 21; fig. 10, pl. 22), sometimes free, sometimes within the cytoplasm and partly enveloped by the nucleus. The inclusions are often surrounded by a light zone as though they were partly digested and absorbed (fig. 3B, pl. 20); indeed, vacuoles, instead of solid masses, in similar situations are not uncommon (V, figs. 11 and 12B, pl. 22).

Blastocyst No. 189' (12) is worthy of special notice. It appeared to be normal in every respect, and the embryonic area, which measures 0.75 mm., was dissected off and stained and mounted intact in balsam. The interesting feature of this vesicle is the large number of these dark bodies that are mostly observed in connection with the entodermal cells. The majority of these cells underlying the embryonic area are each provided

with large or small masses, about which the nucleus lies as if about to engulf it. In figure 11, plate 22, are several typical cases drawn with the aid of the camera lucida. At *A* two bodies are found in connection with a single nucleus; and a similar case is seen in section of a somewhat younger blastocyst at *A*, figure 10. At *B*, figure 11, the entodermal cell behaves toward a vacuole as toward a solid mass, a phenomenon by no means rare. The large cell at *C* seems to have completely ingested a mass, the body in the center being not a nucleolus, but a typical inclusion like those marked *Y* in the other figures.

I have looked through the whole series of stages from the first appearance of entoderm to the largest bilaminar blastocyst and find that the foreign bodies just described are present in nearly all cases, whether in total preparations or in serial sections. I am convinced that they are only remnants of undigested yolk. If one recall the young blastocyst containing 40 or 50 entodermal cells (litter No. 356) at a stage when the trophoblast has become considerably attenuated (pl. 17) one notes that the included yolk is almost entirely confined to the embryonic area. So in succeeding stages, numerous granules of yolk are found among the embryonic, seldom within the trophoblastic, cells. More such granules are, with some exceptions, found in the younger than in the older blastocysts. Thus, while the albumen melts away before the embryonic area of the young bilaminar blastocysts, the yolk granules maintain their identity in small rounded masses for a longer time.

f. Mesoderm formation initiated

With the appearance of the first mesodermal cells about six days have elapsed since ovulation, about five days since the beginning of cleavage, or about one-half of the period of gestation, which I am tentatively stating to be ten days in the opossum. The formation of the mesoderm will be treated in the next number of these studies.

SUMMARY

1. Several thousand eggs were removed from several hundred pregnant and pseudopregnant animals during the collecting seasons 1914 to 1917, an average of 11.5 per litter, or 23 from each animal.

2. At least one-third of the average litter of eggs are unfertilized or abnormal (table 1).

3. Six hundred and forty-one normal eggs, from tubal ova to the bilaminar blastocyst, form the basis of the present study.

4. Collection of embryological material from the opossum has become greatly facilitated because of the discovery that the mammary glands of this animal hypertrophy at the approach of ovulation, so that the sexual condition of the female may be predicted with a high degree of certainty, without sacrificing the animal or without loss of time and effort, by simple though trained and practiced palpation of the glands. But the behavior of the mammary glands as well as the other reproductive organs is the same in the early stages of pseudopregnancy and in pregnancy. Ovulation is always spontaneous.

5. A series of photomicrographs of eggs in the living state is presented in the plates 1 to 11.

The development of ten litters for given periods of time is shown in plates 1 and 2 and in figures 1 and 4, plate 9.

Plates 12 and 13 are intended to serve as a résumé of the stages covered by the present study.

The development of the opossum egg is illustrated in one series by the photographic plates 3 to 10 and in another series by the drawings plates 14 to 22.

Six hundred different opossum eggs are shown, including 240 illustrations of some 180 different preparations.

6. The rate of development was determined in a number of cases in which the eggs of the right uterus were allowed to develop a given period of time after the removal of the left uterus and its contents. This method contributed in no small part to the success in securing an unbroken series of stages.

7. The stages covered in this paper comprise about the first

half of the ten-day period of gestation. More exact figures have not as yet been worked out.

8. The first polar body is given off in the ovary, the second in the Fallopian tube as in other mammals (pl. 14).

9. In the Fallopian tube much albumen and the shell membrane are added to the ovum. It probably requires about twenty-four hours for the passage of the egg to the uterus.

10. The haploid number of chromosomes in the opossum is twelve (pl. 14).

11. At no stage in the unsegmented egg is there any evidence of polarity in the distribution of the yolk, as in *Dasyurus*, the bat and some other mammalian eggs, although in the opossum the yolk is abundantly present (pls. 13 and 14).

12. The egg varies considerably in size, but on the average it is about 0.12 mm. in diameter through the ovum and 0.6 mm. through the shell membrane. Some normal eggs attain the diameter of 0.73 mm., owing to the larger amount of albumen deposited (compare fig. 2, pl. 4, and fig. 3, pl. 5).

13. As in probably all marsupials, the egg reaches the uterus unsegmented, hence at an earlier stage than in any of the Eutheria.

14. The pronuclei at first occupy a yolk-free area at the periphery of the egg; then migrate to the yolk-free central portion, where the first cleavage spindle is later to be seen (figs. 20 and 21, pl. 14).

15. Deutoplasmolysis or elimination of yolk begins at the pronuclear stage, continues at the 2-celled stage, and reaches its maximum during the second cleavage (pls. 3 and 15).

16. The quantity of yolk and surrounding cytoplasm extruded varies greatly, hence the size of the blastomeres varies in inverse ratio to the extent of deutoplasmolysis (pl. 15).

17. Deutoplasmolysis occurs by elimination of masses of various sizes on all sides of the egg, not at any particular spot or pole, as in *Dasyurus* and the bat (fig. 4, pl. 3).

18. The two blastomeres of the 2-celled stage are usually of the same size, shape and structure, or they may differ in size. This difference is probably due chiefly to the difference in the amount of yolk extruded (text fig. 4).

19. One blastomere sometimes anticipates the other in division, and as a result 3-celled eggs are found, but not nearly in as large numbers as eggs in the 4-celled stage.

20. The second cleavage plane is at right angles to the first and the spindles in the two cells lie parallel; but the shifting of the blastomeres soon begins, so that in the 3-celled stage the crossed arrangement may already be attained (*K* and *L*, text fig. 4).

21. The crossed arrangement of the blastomeres in the 4-celled egg is, therefore, in the opossum not due to the direction of the cleavage planes in the second cleavage, but is secondarily caused by the shifting of the blastomeres (compare *B* and *D*, text fig. 4).

22. The shifting of the blastomeres is not due to mutual pressure, for in many 4-celled eggs the blastomeres are very small and not even in contact (figs. 6 and 7, pl. 3).

23. There is no morula stage in the marsupials. The blastocyst cavity is virtually present in the 4-celled egg as the space between the blastomeres. In the 16-celled stage, or earlier, in the opossum, the blastomeres have migrated to the periphery and have applied themselves to the zona pellucida. The structure of the blastocysts is clearly indicated. The extruded yolk now lies within the cavity (pls. 4 and 15).

24. The blastocyst wall is usually fully formed at about the 32-celled stage, when all the gaps between the cells are closed by the flattening and multiplication of the cells of the late cleavage stage. This marks the end of cleavage as such, which requires nearly thirty hours of development (fig. 1, pl. 16).

25. During cleavage the only evidence of polarity lies in the difference in the rate of division among the cells at the two poles. The more rapidly dividing cells are probably embryonic and arise from one of the first two blastomeres (text fig. 5).

26. Definite polarity is established at about the 60- to 70-celled stage with the first appearance of the entoderm. One litter at this stage was found six days after copulation, doubtless a case of retarded ovulation, as a later stage was to have been expected (pl. 16).

27. The entoderm arises from entoderm mother cells of very characteristic appearance. They are cells in the blastocyst wall which round up and usually roll out of their place, as it were, into the blastocyst cavity, as in certain invertebrates, or they may remain attached to the wall for some time, in either case multiplying by mitotic division (pls. 7 and 16).

28. The entoderm mother cells all arise from one-half of the egg, the future embryonic area (figs. 15 to 22, pl. 16).

29. The area that remains free of entoderm mother cells is the trophoblastic area; it soon begins to thin and spread out so that the growth of the ovum now begins. Growth is, therefore, at first due to the spreading of the trophoblastic area (pls. 16 and 17).

30. Since the entodermal cells spring from the superficial epithelial layer in the embryonic area, this would better be termed embryonic entectoderm.

31. When the blastocyst has attained a diameter of 0.3 to 0.35 mm., the entoderm is several cells deep, being crowded into a mass which superficially somewhat simulates an Eutherian inner cell mass in the process of spreading. In the opossum only the superficial cells are embryonic ectoderm, all the rest are entodermal (figs. 13 to 15, pl. 17).

32. Such a stage was removed from an animal four days after copulation, or about a day and a half after the beginning of cleavage.

33. The superficial layer of cells is never transitory; it is embryonic ectoderm and not Rauber's layer; it is in active state of mitosis throughout. Rauber's layer is homologous with the non-embryonic or, as Hill has expressed it, the 'trophoblastic' area.

34. The proliferation of entoderm is at an end when the blastocyst has attained a diameter of 0.45 to 0.5 mm., when the trophoblastic area has attained its greatest degree of attenuation (pl. 18).

35. The entoderm now spreads by an active migration of the flattened, definitive entodermal cells toward the opposite pole of the egg (pl. 18).

36. When the spreading is well under way, the blastocyst, previously spherical and centrally placed, is usually flattened like a thick biconvex lens at one pole of the egg, with the embryonic area in contact with the shell membrane (figs. 1 and 2, pl. 6; fig. 4, pl. 10; pls. 12 and 19).

37. Eggs in which the entoderm has just become closed at the lower pole, and which are thus in the beginning of the bilaminar stage, are still about the same size as in the cleavage stages, but the albumen has become more dense and the shell membrane thicker and more resistant (figs. 14 and 17, pl. 13). The albumen disappears with the growth of the blastocyst and egg (pl. 13).

38. The bilaminar blastocyst is simply a double-walled sac consisting of ectoderm without and entoderm within. The two layers are closely applied to each other and to the shell membrane and albumen, and any variation from this condition is due to shrinkage or to abnormality of the egg. There is no 'perivitelline' space in the normal opossum egg, but frequently occurs in abnormal material (pls. 19 and 20).

39. The bilaminar stage is the period of growth, little differentiation occurring until near the first appearance of mesoderm in vesicles 1.5 to 1.8 mm. in diameter (pls. 19 to 22).

40. The 1-mm. stage was once found seven and one-half days after copulation (litter No. 343, fig. 5, pl. 2); the mesoderm first appears about eight hours later (compare litters 343', 346, 346', 353, 353'); the 0.8-mm. stage was once removed about five days after the beginning of cleavage (litter No. 306', fig. 17, pl. 13; figs. 1 and 1A, pl. 21), and 1.4-mm. blastocysts were found at about four and a half days after the beginning of cleavage (compare litters Nos. 191 and 193, figs. 1 and 9, pl. 22).

41. The embryonic area grows in extent with the growth of the egg, so that in the later bilaminar stage its diameter is about one-fifth to one-fourth of the circumference of the egg (compare pl. 18 and figs. 1 to 3, pl. 21, with figs. 1 to 4, pl. 22).

42. As the egg develops, the embryonic area becomes increasingly more sharply set off from the trophoblastic area (fig. 9, pl. 21). The embryonic ectoderm becomes thicker, the cells

cubical to columnar and more densely granular, whereas the trophoblastic ectoderm remains flat and its cytoplasm reticular (pl. 21).

43. The entoderm in the early stages is everywhere the same, consisting of the typical squamous cells with swellings at the nuclei. In surface view the flatter entodermal nuclei, as a rule, appear larger than the ectodermal. The entoderm nowhere comes to the surface of the blastocyst (pls. 10 and 19 to 22).

44. In blastocysts over 1 mm. in diameter the entoderm is often modified at one side of the embryonic area. These cells increase in number, thickness of nuclei, and density of the cytoplasm, and I believe them to mark the future posterior, not the future anterior margin of the embryonic area. The primitive streak will be laid down here (*ENT*, fig. 9A, pl. 22).

45. These eggs also exhibit a clear field a little to one side of the center in the embryonic area (figs. 1 and 2, pl. 10; fig. 9, pl. 22). This is due to a thinning out of the embryonic ectoderm. Where the light field comes nearest to the margin is the posterior margin of the area, for here the modified entoderm is also found (pl. 22).

46. Yolk spherules occur in the bilaminar stage even in the largest specimens. They are remnants of the extrusions of cleavage stages. They are found often within the cells, usually of the embryonic area only, both ectodermal and entodermal, and the nuclei frequently surround the masses as if to engulf them (figs. 9B, 10 to 12, pl. 22).

47. The egg of the opossum is like that of *Dasyurus* in its possession of a large amount of yolk, in the absence of the morula stage and in the formation of entoderm from entoderm mother cells coming from the wall of the unilaminar blastocyst. But it differs in many regards: in the absence of polarity, in the uniform distribution of the yolk, and in the consequent manner of deuteroplasmolysis; in the indeterminate type of cleavage; in the crossed arrangement of the 4-celled egg; in the early period in which the blastocyst is formed; in the very early formation of the entoderm; in the simulation of an inner cell mass due to the crowding of the primitive entoderm cells,—in these respects

the opossum egg is much more Eutherian than Dasyurine. Of these perhaps the most striking feature is very early differentiation of the entoderm. Since in other marsupials, according to Hill, the entoderm is formed early (*Macropus*, *Perameles*) it seems probable that when the other marsupials have been more thoroughly studied it will be found that the opossum is more typical of the marsupials in general and that *Dasyurus* represents a more primitive, albeit, therefore, an even more interesting form.

ADDENDUM

Since the completion of my manuscript there has come to hand Prof. J. P. Hill's paper on "The Early Development of *Didelphys aurita*," published in the April, 1918, number of the *Quarterly Journal of Microscopical Science*. This work is based on eggs secured from six females: one animal furnished unsegmented, unfertilized eggs; another numerous 2-, 3-, and 4-celled eggs; from two others, cleavage stages of 4 to 16 cells were taken, and from two animals bilaminar blastocysts about 1 mm. in diameter.

It appears from this contribution that the developmental stages of the South American opossum, so far as Professor Hill's material goes to show, are closely duplicated by my own specimens of the local species. The same is true of a number of somewhat later stages (primitive streak) which I have secured from the small black *D. marsupialis* occurring in south Texas and Mexico.

In most respects my own work finds full corroboration as well as interesting extensions in the careful study made by Professor Hill. I wish briefly to refer to several points discussed by our able British colleague.

In his analysis of the 2-, 4-, 8-, and 16-celled stages, he presents additional evidence of polarity in the opossum egg; for he finds that in a large proportion of such eggs the cells are plainly made up of two groups, differing somewhat in size, the smaller cells being considered by Hill as constituting the upper or formative pole of the egg. He also finds that the majority of eggs in

later cleavage show an accelerated rate of division at one pole. The evidence of polar differentiation in the opossum egg throughout cleavage seems, therefore, to be complete. Professor Hill recognizes fully the difference between the cleavage of the opossum and of *Dasyurus* and joins me in deriving the formative and the non-formative areas each from one of the two blastomeres of the 2-celled egg.

As to the method of deutoplasmolysis, Hill considers that "the yolk spheres are budded off from a narrow, clear zone which has made its appearance at the exposed surfaces of the blastomeres" and his "figures shown undoubted yolk spheres in direct continuity with the lighter peripheral zone." I have noted the same phenomenon, although never as pronounced as in the cases illustrated by Hill in his figures 11 to 13, plate 8. In most of my hundred specimens, the smooth, unwrinkled cell membrane can be followed clearly around the blastomeres. Professor Hill is correct in assuming that the egg No. 50 (6) upon which I based my former conclusion on the method of yolk elimination (Hartman, '16, page 23, and fig. 9, pl. 5) is probably not quite normal; in fact, the specimen was considerably retarded in development as compared with its fellows in the 50 to 70-celled stage. Such retarded eggs are always to be regarded with suspicion. I therefore no longer regard yolk elimination as due to the "formation of a new cell membrane, . . . at a distance from the original surface of the blastomeres," but believe with Professor Hill that masses of variable size are extruded from different places on the exposed surfaces of the blastomeres.

On the origin of the crossed arrangement of the blastomeres in the 4-celled egg, Hill presents evidence, which taken by itself, would be conclusive of the fact that the blastomeres do not attain this position by shifting, but assume it from the beginning by virtue of a meridional division of one blastomere and an equatorial division of the other. For out of his ten 2-celled eggs, five have both blastomeres in the process of division and in these the axes of the blastomeres are already nearly or quite at right angles to each other. Three of these eggs have completed their

nuclear division, the nuclei being in the resting stage; the other two are in late anaphase.

However, two of my own specimens, both of which contain short spindles in each blastomere, cast doubt upon Hill's view as stated above, with which I had agreed before I came into possession of the eggs (from No. 306). For in one of these eggs the spindles are exactly parallel; in the other they deviate 36° from the parallel.

It is, therefore, apparent that, unless we assume a rapid shifting of the blastomeres during the early phases of the second cleavage, the matter must for the present remain in doubt.

It is interesting to note that *D. aurita* has two breeding seasons a year, whereas *D. virginiana* has but one.

Professor Hill states it as his belief that twelve is the reduced number of chromosomes in the opossum, and with this I fully agree.

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Plates 1 to 11 contain 82 figures nearly two-thirds of which are from photographs of living eggs. Plates 1 and 2 show the development of nine litters for given periods of time. Plates 3 to 10 are arranged by stage of development, and the same stages are shown in drawings on plates 14 to 22. Plate 12 was drawn from 8 specimens as they appeared in the living state. Plate 14 is a résumé of the stages covered in this paper as drawn from sections.

PLATE 1

EXPLANATION OF FIGURES

Photomicrographs of living eggs in Ringer's solution; the ten figures show two litters from each of five different animals; figs. 1 to 9 $\times 8$; fig. 10, natural size.

- 1 Litter No. 320; 4-celled eggs.
- 2 Litter No. 320'; interval $5\frac{1}{2}$ days; primitive-streak stage.
- 3 Litter No. 299; 4-celled eggs.
- 4 Litter No. 299'; interval 4 days, $3\frac{3}{4}$ hours; blastocysts partly bilaminar.
- 5 Litter No. 292; young unilaminar blastocysts containing 40 to 50 cells.
- 6 Litter No. 292'; interval 4 days; primitive-streak stages.
- 7 Litter No. 307; tubal ova with a little albumen on one side.
- 8 Litter No. 307'; interval $5\frac{3}{4}$ days; unfertilized, fragmenting eggs; albumen rather opaque.
- 9 Litter No. 337; 8- to 16-celled eggs.
- 10 Litter No. 337'; vesicles with primitive streak and very short medullary groove; photographed in open uterus, natural size.

GENERAL ABBREVIATIONS

- | | |
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| <i>A</i> , designates particular portions of various drawings to which reference is made in the text | <i>ENT</i> ² , undifferentiated primitive entodermal cells (pls. 17 and 18) |
| <i>ALB</i> , albumen | <i>ENT</i> ³ , flattened definitive entodermal cells (pl. 16) |
| <i>ART</i> , artifact | <i>GR</i> , granulosa cells of discus proligerus |
| <i>C</i> , coagulum | <i>O</i> , 'blister' in trophoblastic ectoderm |
| <i>CH</i> , chromosomes | <i>PB</i> , polar body |
| <i>EMB.A</i> , embryonic area | <i>SM</i> , shell membrane |
| <i>EMB</i> , <i>ECT</i> , embryonic ectoderm or entectoderm | <i>TR.A</i> , trophoblastic or non-embryonic area |
| <i>ENT</i> , definitive entoderm; in plate 18 it designates the limit of spread of entoderm; in plates 21 and 22 it refers to certain specialized entodermal cells | <i>TR. ETC</i> , trophoblastic ectoderm |
| <i>ENT</i> ^A , entoderm mother cells in wall of blastocyst (pls. 16 and 17) | <i>V</i> , vacuole |
| <i>ENT</i> ¹ , entoderm mother cells that have migrated into cavity of blastocyst (pls. 16 and 17) | <i>X</i> , placed at limits of embryonic area (junctional line) |
| | <i>XX</i> , embryonic area of sections, junctional line of surface views |
| | <i>Y</i> , yolk masses |
| | <i>ZP</i> , zona pellucida |

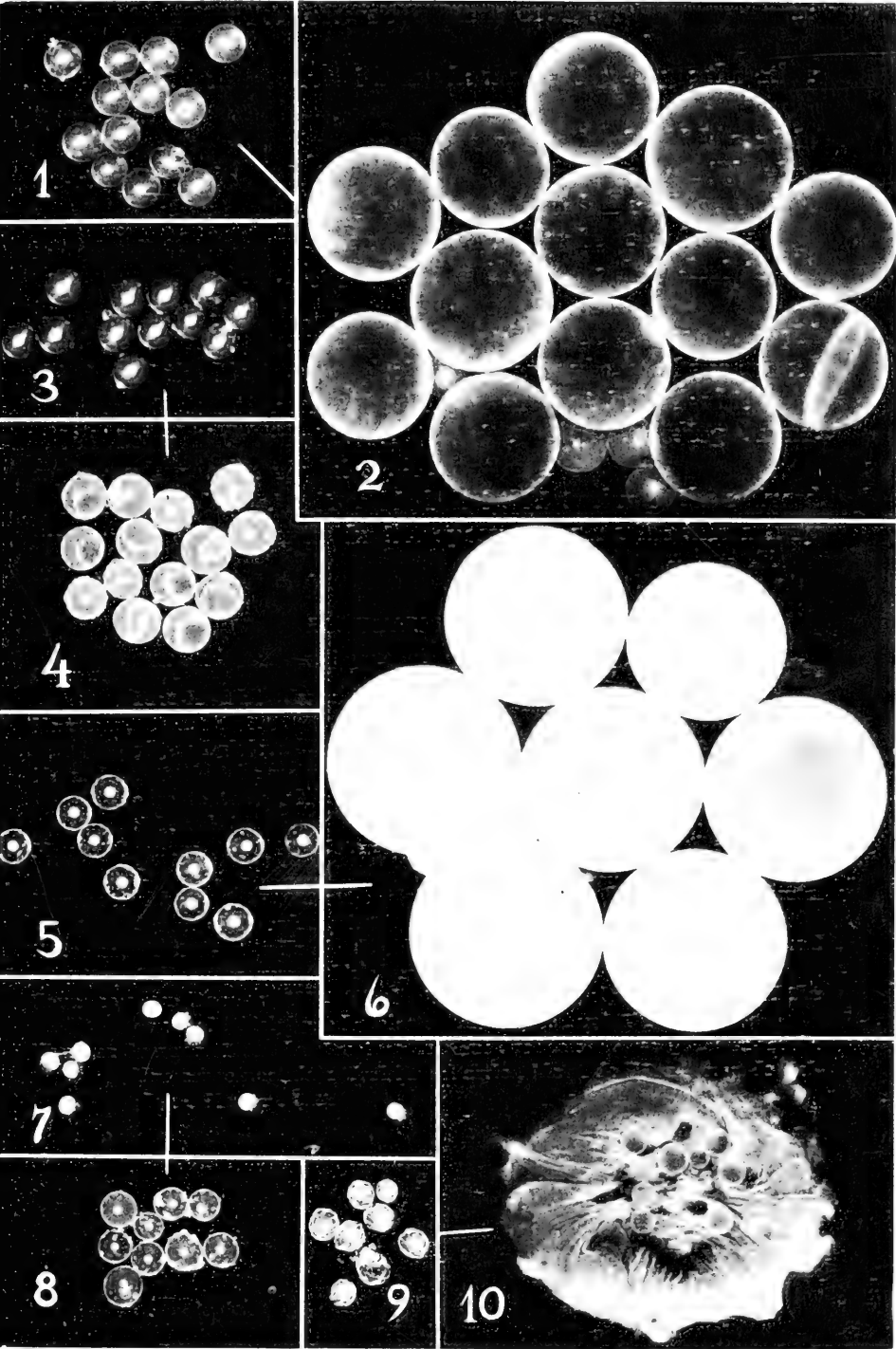


PLATE 2

EXPLANATION OF FIGURES

Photomicrographs of living eggs; in Ringer's solution by reflected light; in the eight figures two litters are shown from each of four animals; all figures $\times 8$, except fig. 8, which is $\times 2$.

- 1 Litter No. 293; 4-celled eggs.
- 2 Litter No. 293'; interval $3\frac{1}{2}$ days; young bilaminar blastocysts.
- 3 Litter No. 346; bilaminar blastocysts.
- 4 Litter No. 346'; interval $9\frac{3}{4}$ hours; early primitive-streak stage.
- 5 Litter No. 343; 1 mm., bilaminar blastocysts.
- 6 Litter No. 343'; interval 7 hours and 20 minutes; bilaminar blastocysts just preceding first proliferation of mesoderm.
- 7 Litter No. 298; unilaminar blastocysts of 60 to 120 cells with entoderm mother cells.
- 8 Litter No. 298'; interval $3\frac{1}{2}$ days; vesicles in opened uterus; primitive streak and short medullary groove. $\times 2$.

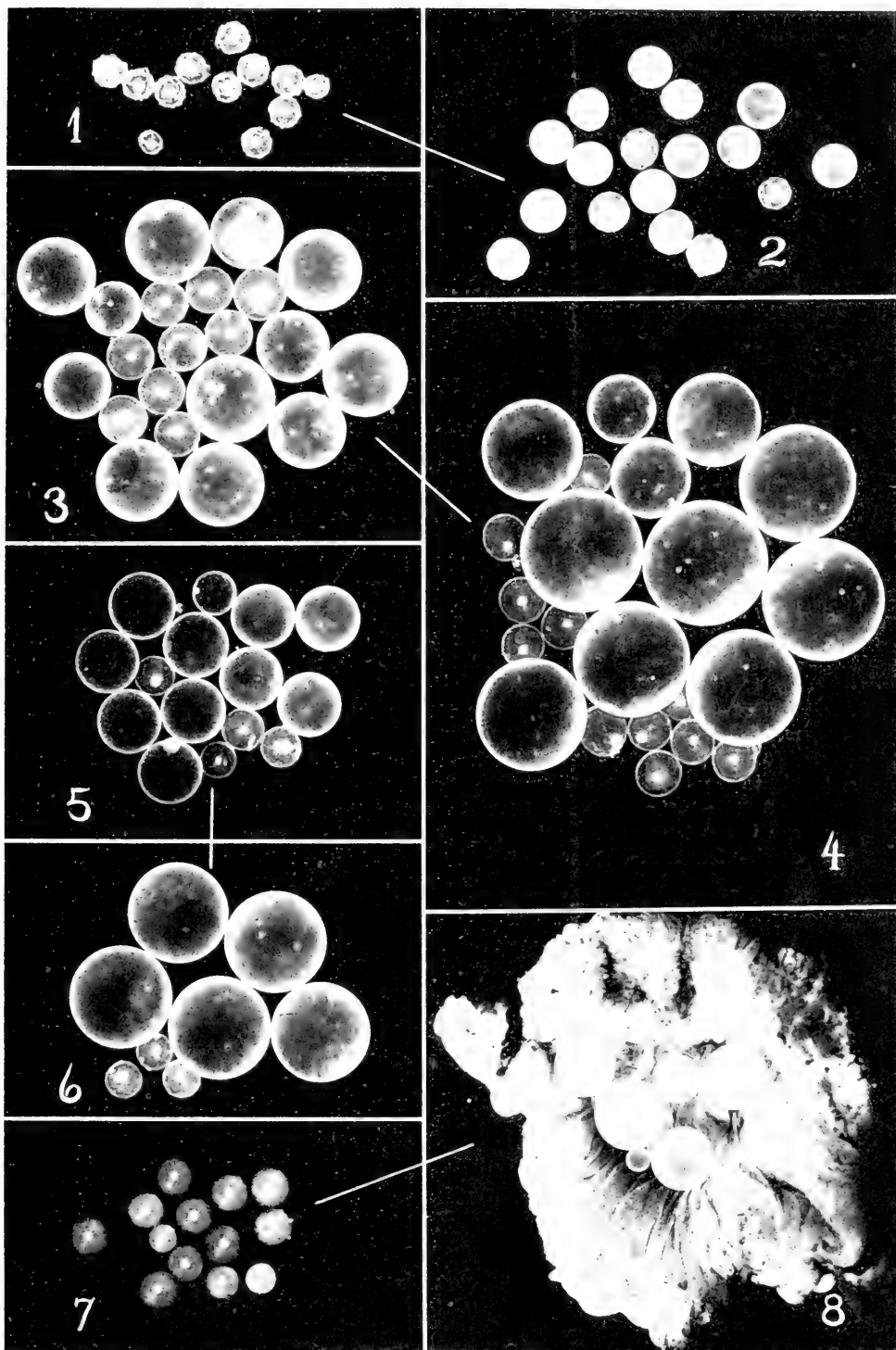


PLATE 3

EXPLANATION OF FIGURES

Photomicrographs of tubal ova and early cleavage stages in uterine eggs.

1 Two eggs of litter No 313, photographed in the living state in Ringer's solution by reflected light; considerable albumen has been deposited. $\times 130$.

2 Litter No. 351', photographed in Ringer's solution by transmitted light; a small ring of albumen is seen. $\times 56.5$.

3 Section through ovum No. 313 (4); 10th section (total in series 19); polar body shown above; the albumen is darkly stained with Delafield's haematoxylin; yolk stained with osmic acid; Hill's fluid; 5μ . $\times 200$.

4 2-celled ovum No. 306 (2); 10th section (total 21); compare C and D, text fig. 4; Hill's fluid; 5μ . $\times 200$.

5 4-celled ovum No. 173 (5); 11th section (total 19); aceto-osmic-bichromate; 5μ . $\times 200$.

6 and 7 Sections 9 and 14 through ovum No. 299 (5) showing four small blastomeres and much extruded yolk; Hill's fluid; 5μ . $\times 200$.

8 14-celled ovum No. 193 (6); 8th section (total 17); two large cells (of which one is cut longitudinally in section) have undergone nuclear division; aceto-osmic-bichromate. $\times 200$.

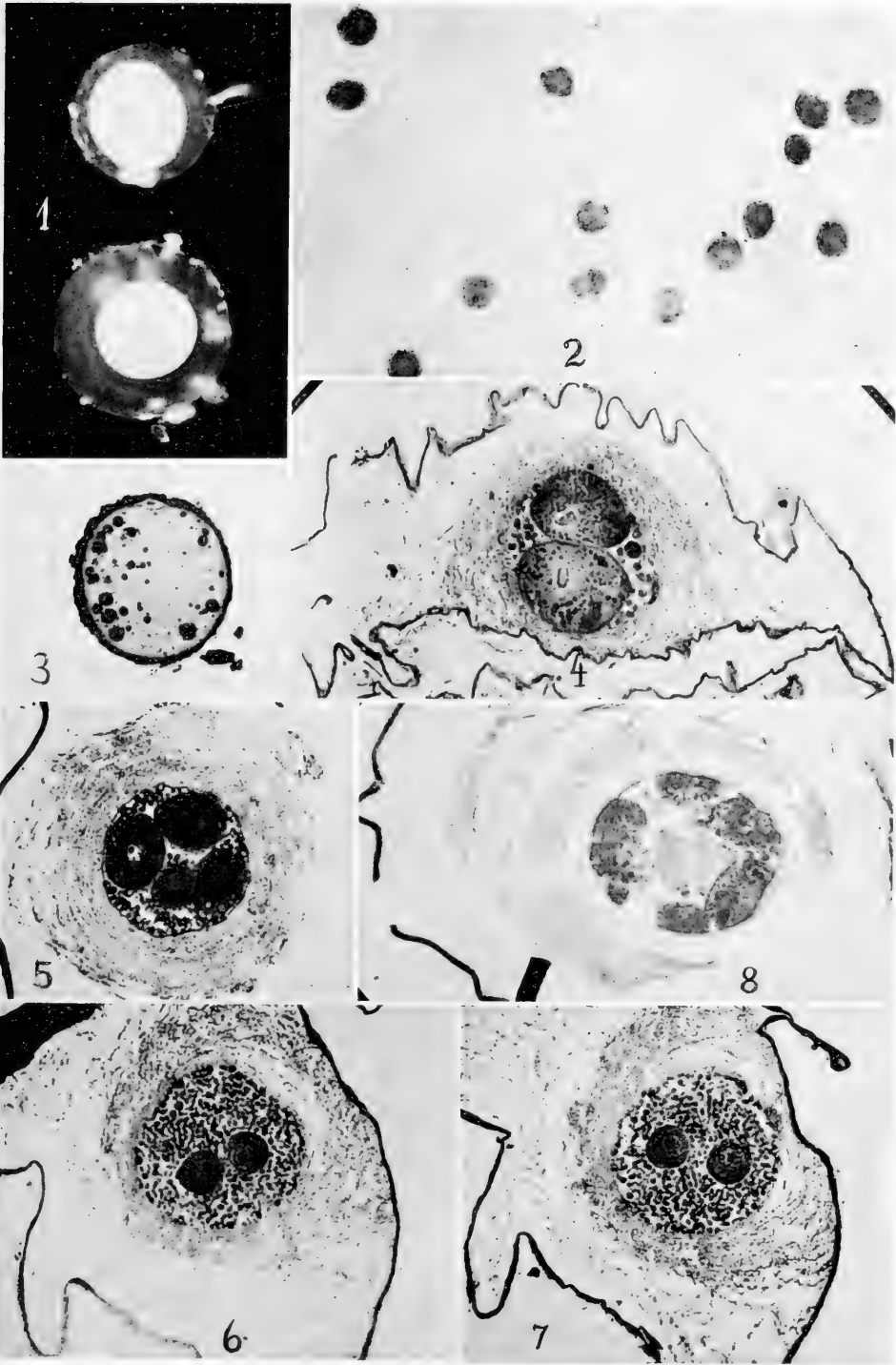


PLATE 4

EXPLANATION OF FIGURES

Late cleavage stage as illustrated by litter No. 336.

1 The living eggs as photographed in Ringer's solution by transmitted light. $\times 36$.

2 Two eggs of the same litter; the peripheral arrangement of the blastomeres is apparent. $\times 82$.

3 32-celled ovum No. 336 (5); blastocyst still incomplete; 9th section (total 20); Flemming; 5μ . $\times 200$.

4 30-celled ovum No. 336 (4); incomplete blastocyst; section taken through middle of ovum (about 19 sections); Flemming; 5μ . $\times 200$.

5 The eggs of litter No. 336 photographed by reflected light in Ringer's solution. $\times 8$.

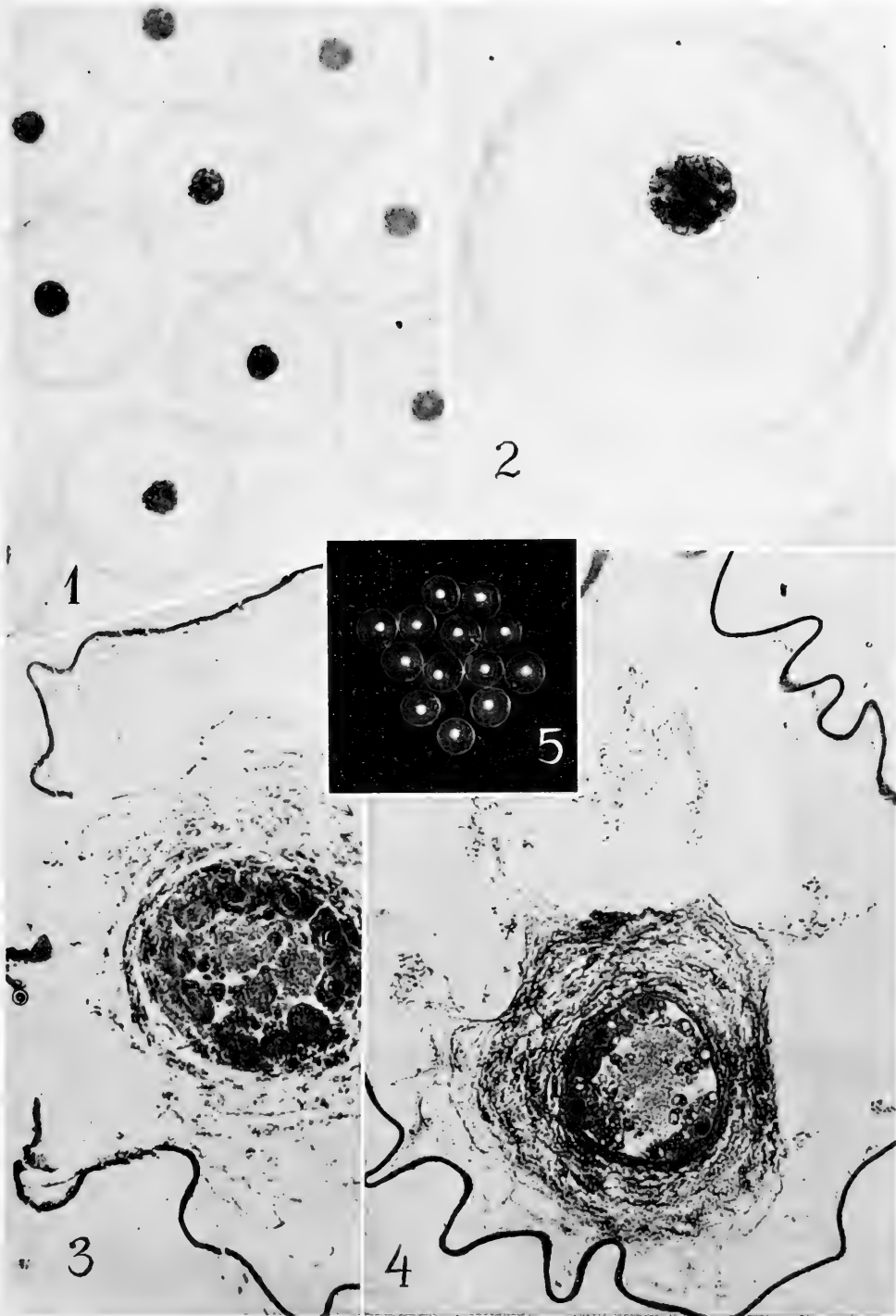


PLATE 5

EXPLANATION OF FIGURES

Photomicrographs of late cleavage stages; all figures except 2 and 5 were photographed in the living state in Ringer's solution; figs. 1 and 7 by reflected light; figs. 3 and 4, by transmitted light.

1 Litter No. 314. $\times 8$.

2 Ovum No. 314 (2); 13th section (total 19); 28 cells in the incomplete blastocyst wall and 2 cells in cavity, of which only one cell is shown; the cells are highly vacuolated; Bouin; 5μ . $\times 200$.

3 Several eggs from litter No. 337; about 16-celled stage; the peripheral arrangement of the blastomeres is well seen; compare fig. 4, below, and fig. 9, pl. 1. $\times 82$.

4 Litter No. 337; one egg with the albumen has been removed from its shell membrane. $\times 36$.

5 Several eggs in cleavage stages and young blastocysts, stained in Delafield's haematoxylin and photographed in oil of wintergreen; the albumen is darkly stained in some cases; see accompanying text fig. 6 for key. $\times 16$.

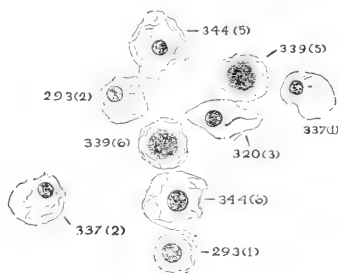


Fig. 6. Key to figure 5, plate 5.

6 One egg of litter No. 342 shown in fig. 7; late cleavage stage, about 26 cells. $\times 36$.

7 Litter No. 342. $\times 8$.

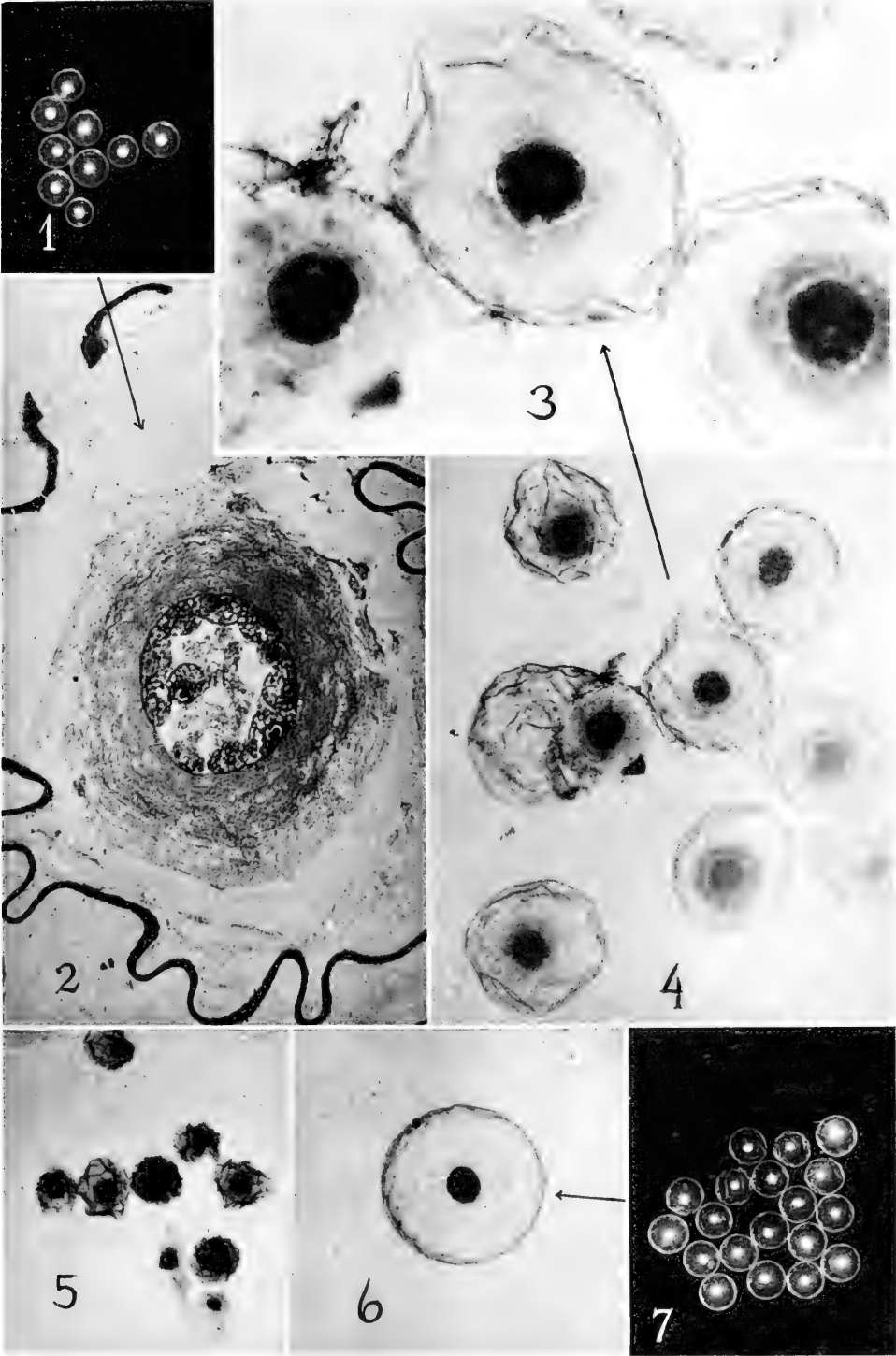


PLATE 6

EXPLANATION OF FIGURES

Mostly unilaminar blastocysts.

1 Group of young unilaminar to early bilaminar blastocysts photographed in oil of wintergreen; fixation mostly by solutions containing osmic acid; for identification of individual eggs see accompanying illustration, text fig. 7. $\times 16$.

2 Group of blastocysts stained in Delafield's haematoxylin and cleared in oil of wintergreen; Bouin; see accompanying illustration, text fig. 8 for key. $\times 16$.

3 Section 13 through ovum No. 88 (7), reconstructed in fig. 21, pl. 16; 18 sections in series; 87 cells, including 5 entoderm mother cells; Hill's fluid. $\times 200$

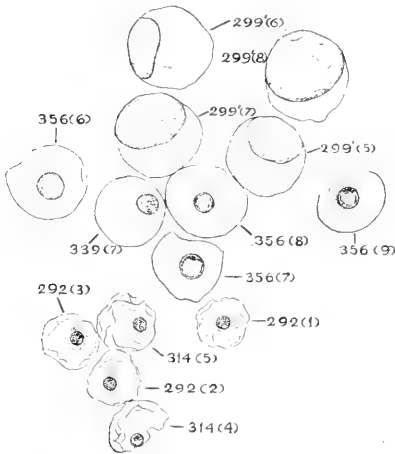


Fig. 7. Key to figure 1, plate 6.

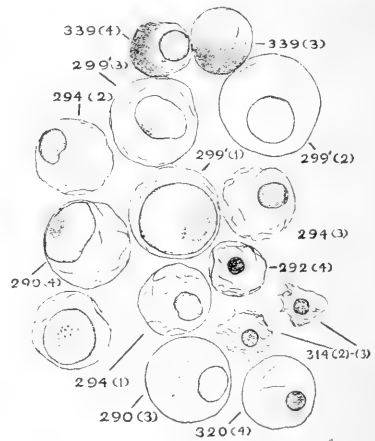


Fig. 8. Key to figure 2, plate 6.

4 Just completed 32-celled blastocyst No. 314 (5), 9th section (total 19 sections); Hill's fluid; 5μ ; compare fig. 1, pl. 5, and fig. 2 above. $\times 200$.

5 Litter No. 292, photographed alive in Ringer's solution. $\times 8$.

6 Section 10 through the 46-celled, incomplete blastocyst No. 292 (4), also seen in fig. 2 above; 19 sections in series; no entodermal cells; Hill's fluid; 5μ . $\times 200$.

7 and 8 Ovum No. 298 (1); fig. 7, whole egg, $\times 30$, in alcohol (compare fig. 7, pl. 2); fig. 8, 6th section showing column of entoderm mother cells; 21 sections in series; 64 cells, including 4 entoderm mother cells; Hill's fluid; 5μ . $\times 200$.

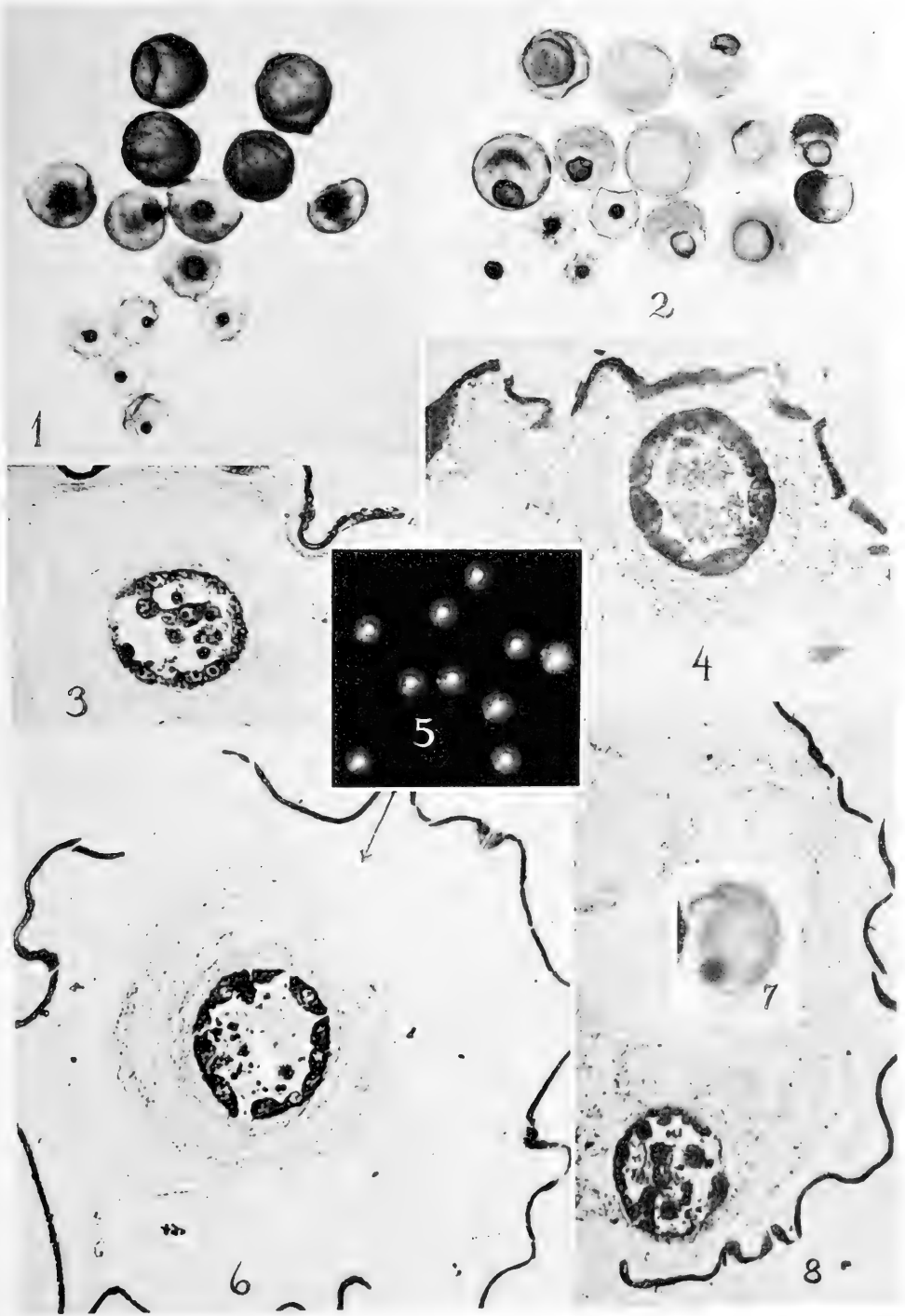


PLATE 7

EXPLANATION OF FIGURES

Mostly blastocysts with entoderm mother cells.

1 Portion of 13th section through ovum No. 50 (5), showing its only entoderm mother cell; total 21 sections; 65 cells; Bouin; 5μ . \times 500.

2 Portion of 13th section through ovum No. 88 (17), taken as indicated by parallel lines on fig. 18, pl. 16; characteristic entoderm mother cell is shown; total 18 sections; 103 cells, of which 6 are entoderm mother cells; Hill's fluid; 5μ . \times 500.

3 Section through 6 of the 9 entoderm mother cells of ovum No. 50 (4); 16th section (total 20 sections); 67 cells; Hill's fluid; 5μ . \times 200.

4 The 15th section through ovum No. 88 (16), of which the 11th section is shown in fig. 11, pl. 16; total 20 sections; large entoderm mother cell is shown in blastocysts wall; 82 cells, including 10 entoderm mother cells; Bouin; 5μ . \times 500.

5 23-celled incomplete blastocyst No. 173' (7); 13th of a total of 23 sections; aceto-osmic-bichromate; 5μ . \times 200.

6 The 12th section through ovum No. 88 (3), showing several entoderm mother cells and much yolk and coagulum; 23 sections in series; 69 cells, of which 10 are entoderm mother cells; Bouin; 5μ . \times 500.

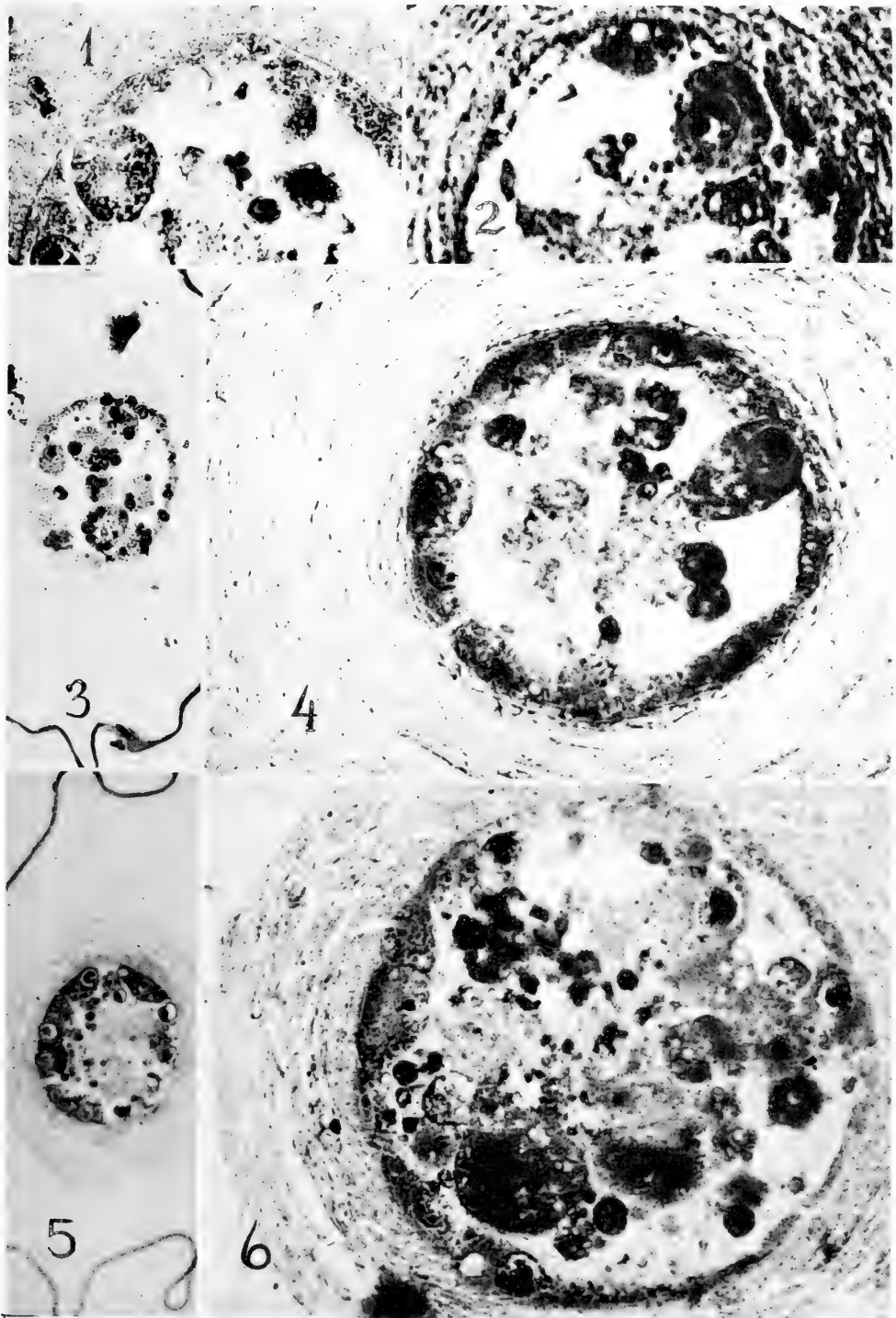


PLATE 8

EXPLANATION OF FIGURES

Photomicrographs showing progress in entoderm formation and polar differentiation; figs. 1, 3, 5, and 6, photographed in the living state in Ringer's solution by transmitted light.

1 An egg of litter No. 356, showing opaque embryonic area and thin trophoblastic area. $\times 82$.

2 Blastocyst No. 356 (7); 16th section (total 32); Flemming 5μ ; $\times 200$; compare with fig. 1.

3 One of two identical eggs from litter No. 349; opaque embryonic area to the left; compare fig. 12, pl. 17. $\times 36$.

4 Egg No. 349 (5), the least developed egg from litter No. 349; polar differentiation is well under way; retarded in development as compared with fig. 3; 14th section (total 25); Bouin, 5μ . $\times 200$.

5 Egg No. 344 (7), lateral aspect, as seen alive; the longitudinal section of this egg is shown in fig. 7. $\times 82$.

6 Egg No. 344 (8), as viewed with opaque embryonic area uppermost. $\times 82$.

7 Egg No. 344 (7), in section, seen alive in fig. 5; 10th section (total 23); Flemming; 5 pl. $\times 500$.

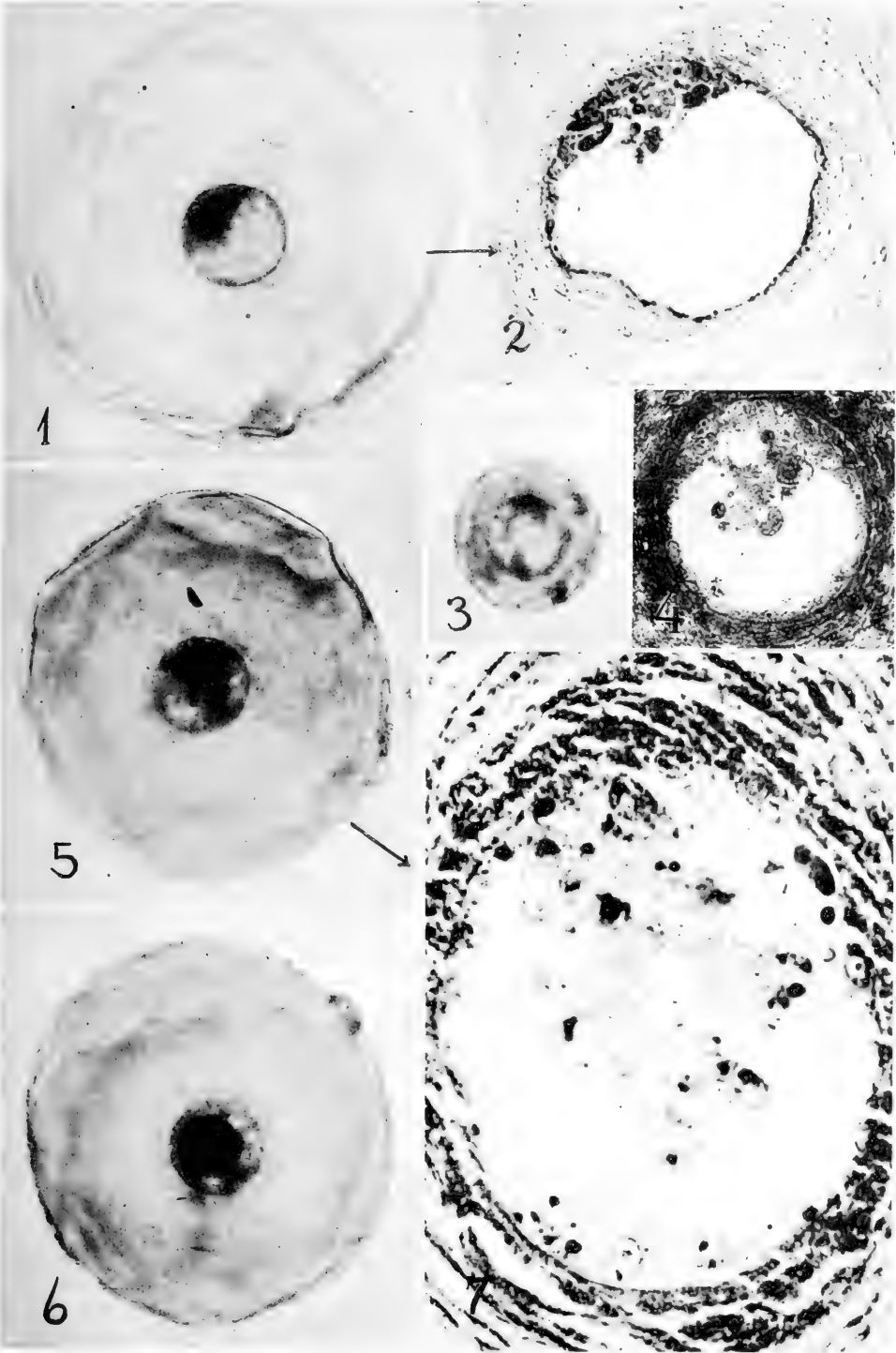


PLATE 9

EXPLANATION OF FIGURES

Photomicrographs of late unilaminar and young bilaminar blastocysts; figs. 1, 4, 5, and 6, photographed alive in Ringer's solution; the first by transmitted light, $\times 36$, the last three by reflected light, $\times 8$.

1 Litter No. 352, blastocysts with bilaminar embryonic area (indicated by dark region at one pole of the vesicle); trophoblastic area very attenuated; compare fig. 5, pl. 12.

2 Section through embryonic area of egg No. 352 (11); 44th section of egg (total 96); 26th section of blastocyst (total 66); 16th section of embryonic area (total 34); entoderm has begun to migrate beyond area; half-strength Bouin; 5μ . $\times 100$.

3 Section 12 of ovum No. 356 (5), of which sections 13 and 18 are shown in figs. 10 and 11, pl. 17; total 21 sections; Bouin; 6μ . $\times 500$.

4 Litter No. 352'; interval 15 hours; compare fig. 1; young bilaminar blastocysts; one egg has two blastocysts.

5 Litter No. 347; partially and entirely completed bilaminar blastocysts.

6 Litter No. 339, a little younger than litter No. 347, shown in fig. 5.

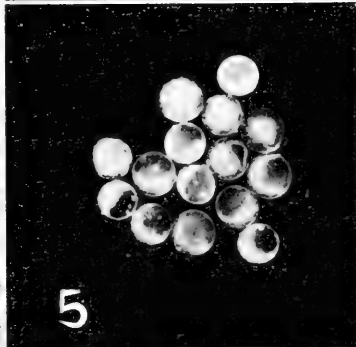
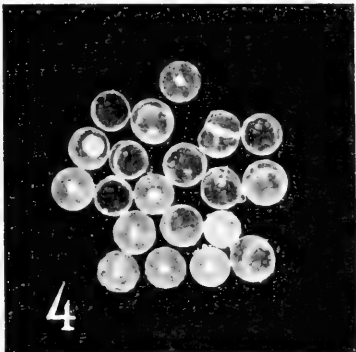
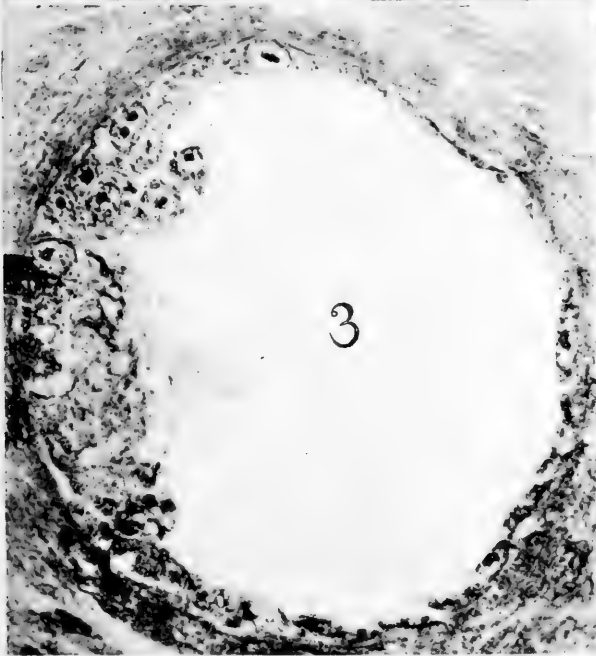
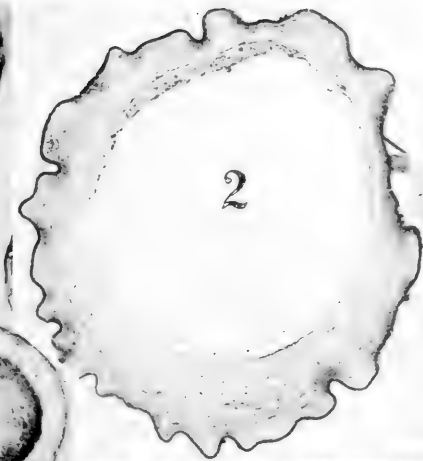


PLATE 10

EXPLANATION OF FIGURES

Photomicrographs of bilaminar blastocysts.

1 and 2 Two eggs of litter No. 193', photographed unstained in alcohol by transmitted light; note light field near center of embryonic area; compare fig. 9, pl. 22. $\times 30$.

3 1.15-mm. blastocyst No. 360 (5), stained in Delafield's haematoxylin, cut in two horizontally and photographed in alcohol by transmitted light. = 16.

4 Egg No. 352' (10), one of the litter shown in fig. 4, pl. 9; 46th section of vesicle (total 92); 29th section of embryonic area (total 54); section is oriented with embryonic area to left; Hill's fluid; 5μ . $\times 100$.

5 Egg No. 299' (6); one of litter shown in fig. 4, pl. 1; shown in toto in fig. 1, pl. 6; 61st section of vesicle (total 120); 41st section of embryonic area (total 81); the entoderm has not quite reached the lower pole; Hill's fluid; 5μ . $\times 100$.

6 Section of egg No. 360 (5) shown in surface view in fig. 3; section is oriented with embryonic area uppermost; a little albumen is left at lower pole; 55th section of embryonic area (total 121). $\times 100$.

7 Litter No. 306'; interval 5 days, $20\frac{1}{2}$ hours, after beginning of cleavage; photographed alive in Ringer's solution. $\times 8$.

8 Stereogram of ova No. 360 (7), (8), and (9); photographed in alcohol; embryonic area seen in two of the eggs; Zenker. $\times 6.3$.

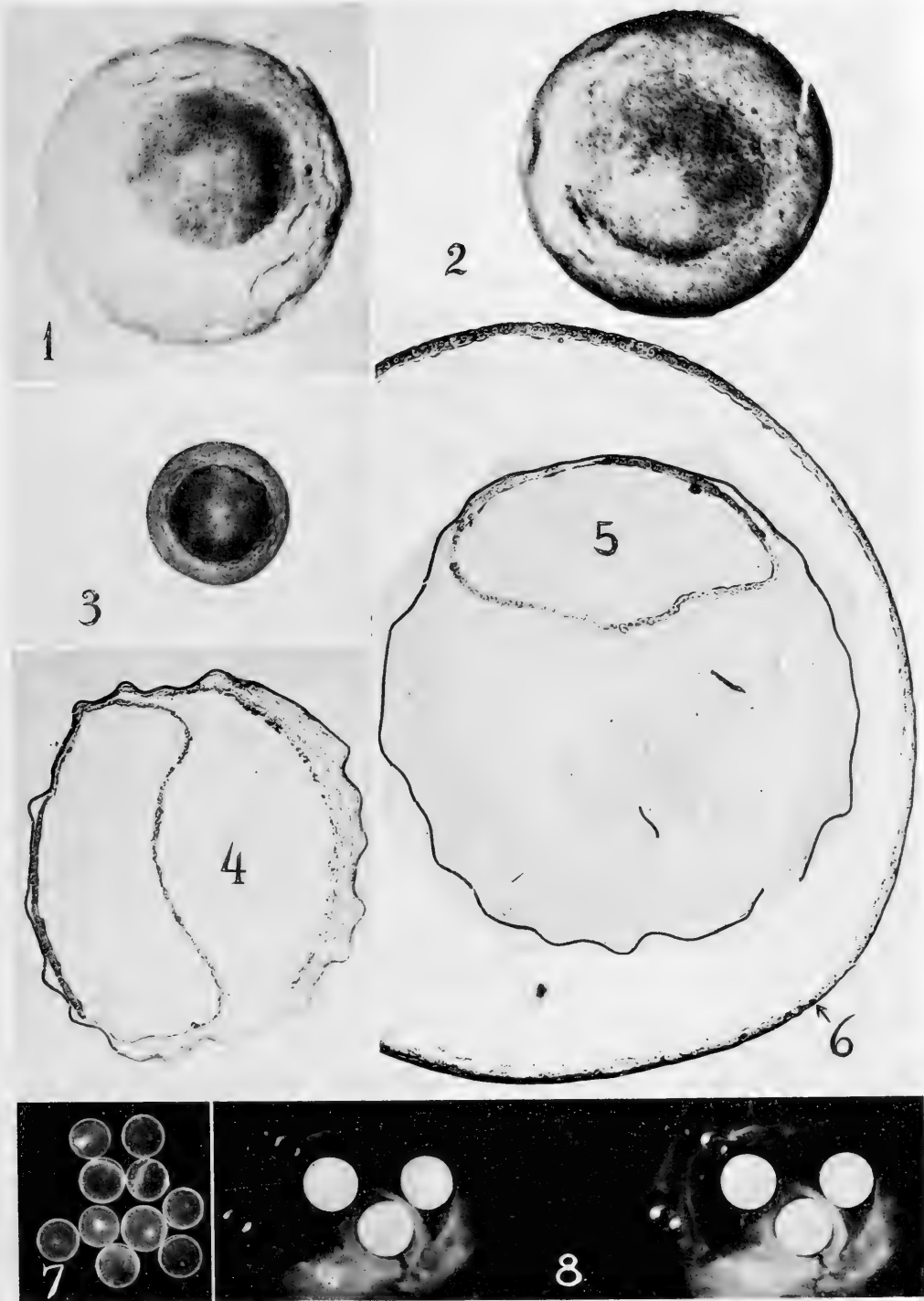


PLATE 11

EXPLANATION OF FIGURES

Photomicrographs taken alive by reflected light in Ringer's solution; except fig. 2. Figs. 1 to 4, bilaminar blastocysts; figs. 5 to 10, unfertilized eggs. Magnification, $\times 8$, except fig. 5.

1 Litter No. 294, mostly abnormal blastocysts with still largely unilaminar walls (compare fig. 2, pl. 6).

2 Part of litter No. 290', photographed a few minutes after immersion in Hill's fixing fluid; embryonic area is well seen.

3 Litter No. 290'; bilaminar blastocysts (compare fig. 2, pl. 6).

4 Litter No. 294' (interval $34\frac{1}{4}$ hours; compare fig. 1); mostly abnormal bilaminar blastocysts.

5 Litter No. 415; unfertilized eggs in early stage of fragmentation; shows false '1-celled,' '2-celled,' and '4-celled' eggs.

6 Litter No. 318; early eggs in fragmentation; note that the ovum proper is no longer spherical.

7 Litter No. 303, with opaque albumen and fragmenting ova.

8 Litter No. 297; old fragmenting eggs with white concretions on shell membrane.

9 Four degeneration eggs that accompanied fetuses one day before birth; Litter No. 321'.

10 Litter No. 332; degenerating eggs nine or ten days old.

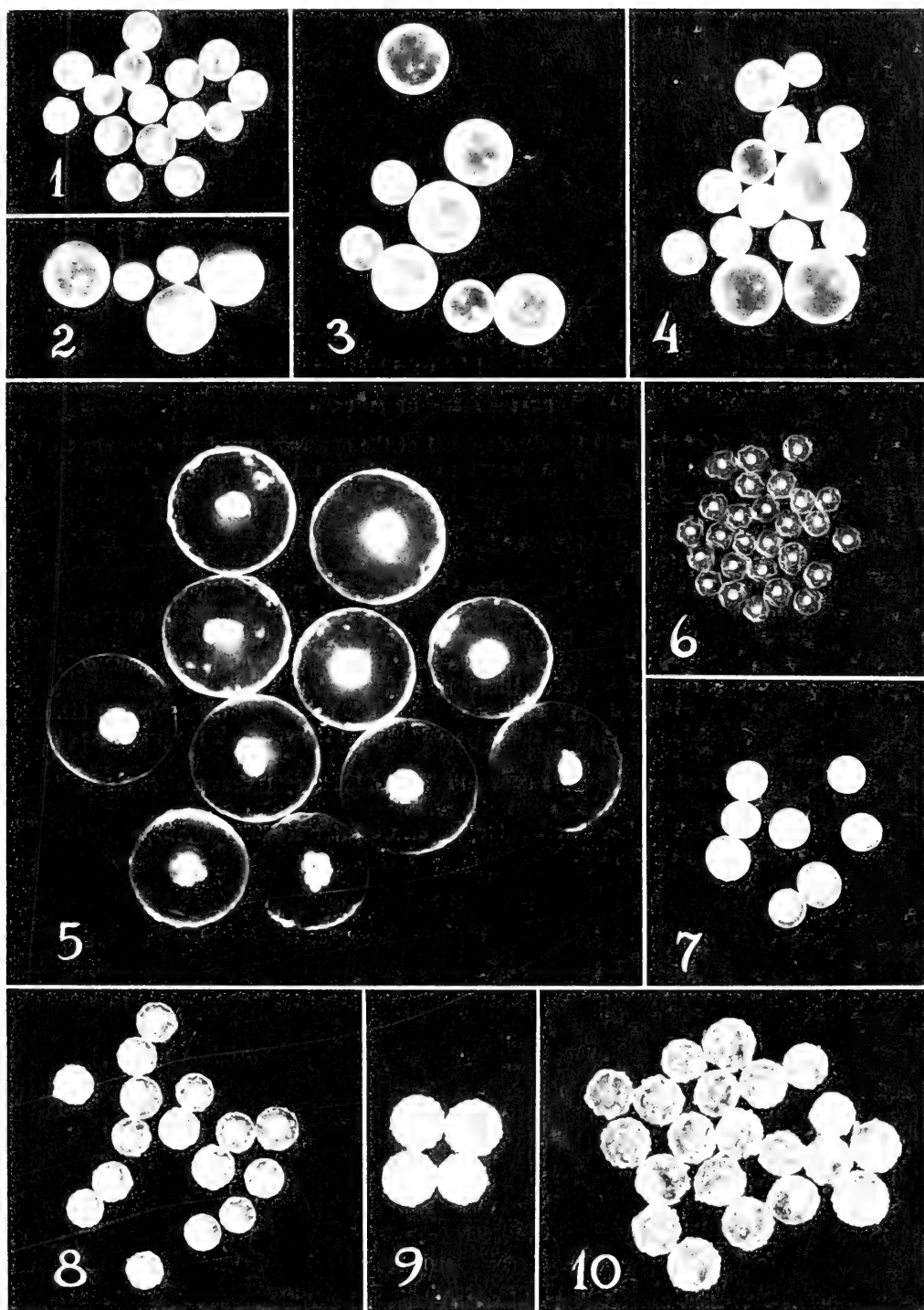


PLATE 12

EXPLANATION OF FIGURES

Résumé of stages in the development of the opossum eggs from cleavage to the completed bilaminar blastocysts; drawn from actual specimens cleared in oil of wintergreen, the measurements being made from photographs of the living egg. $\times 50$.

- 1 The unsegmented uterine egg.
- 2 The 4-celled ovum.
- 3 The just completed unilaminar blastocyst of about 32 cells.
- 4 Blastocyst with polar differentiation well under way; primitive entoderm present; drawn after No. 356 (6) (compare fig. 2, pl. 6).
- 5 Blastocyst with attenuated unilaminar trophoblastic area, bilaminar only in the embryonic region (after litters Nos. 194', 175', and 352).
- 6 More advanced blastocyst with spreading entoderm; after No. 290 (4), photographed in fig. 2, pl. 6; the flattened shape of the vesicle is the usual one at this stage.
- 7 Similar stage with more unusual spherical blastocyst, drawn after No. 299' (2), shown photographically in fig. 2, pl. 6.
- 8 Completed bilaminar blastocyst, drawn after No. 299' (1), shown photographically in fig. 2, pl. 6.

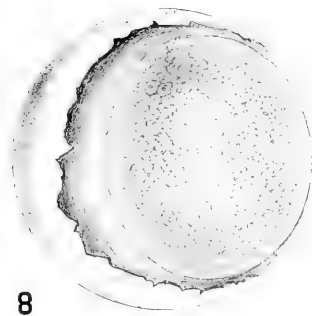
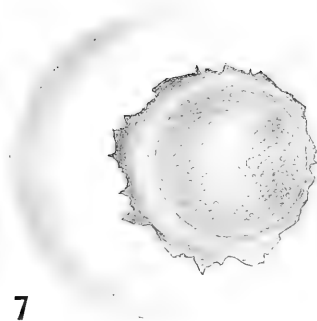
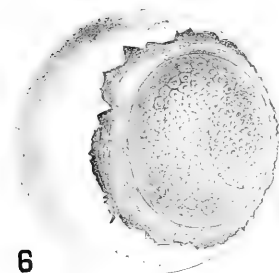
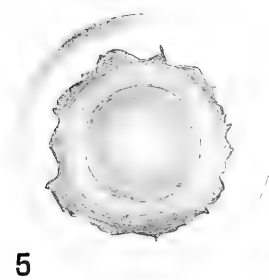
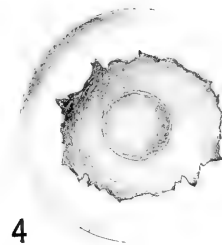
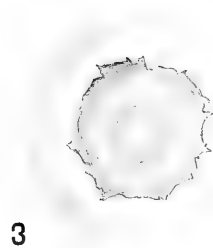
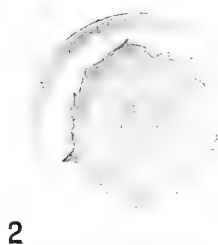
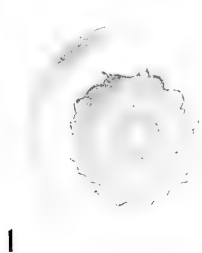


PLATE 13

EXPLANATION OF FIGURES

Résumé of stages in the development of the opossum egg as drawn from sections of representative specimens from the ovarian egg to the primitive streak stage. $\times 50$.

- 1 Ovarian egg from specimen No. 28; Hermann's fluid.
- 2 to 5 Respectively the following tubal ova: No. 76 (8), Hill's fluid; No. 56 (4), Bouin's; No. 351 (1); Hills; No. 313' (1), Bouin's.
- 6 Unsegmented uterine egg No. 287 (1); Hill's fluid; $5\ \mu$.
- 7 2-celled ovum No. 203 (4); thirteenth section (total 20); $5\ \mu$.
- 8 4-celled ovum No. 203 (5); tenth section (total 21); Hill's fluid; $5\ \mu$.
- 9 14-celled ovum No. 193 (6); eighth section (total 17); aceto-osmic-bichromate.
- 10 Just completed blastocyst No. 191 (2); eleventh section (total 18); 32 cells; Bouin; $6\ \mu$.
- 11 63-celled blastocyst No. 50 (8), of which one of the two entoderm mother cells is shown in fig. 5, pl. 16; ninth of 17 sections; Hill's fluid.
- 12 Egg No. 356 (4), the seventeenth section (total 25); compare figs. 6 and 7, pl. 17; $6\ \mu$.
- 13 Blastocyst No. 194' (3); ninth section of vesicle (total 34); fifth section of embryonic area (total 13); aceto-osmic-bichromate (?); $5\ \mu$.
- 14 No. 352 (7); detail in fig. 8, pl. 21, q. v.
- 15 Egg No. 339 (5); thirtieth section of blastocyst (total 65); not perfectly normal; one-half strength Bouin; $5\ \mu$.
- 16 No. 299' (5), shown in toto in fig. 1, pl. 6; sixty-second section of egg (total 122) and thirty-fifth section of blastocyst (total 83); Hill's fluid; $5\ \mu$.
- 17 No. 306' (2), also shown in fig. 2, pl. 20, q. v.
- 18 Bilaminar blastocyst No. 189 (6), the embryonic area of which is shown in fig. 4, pl. 21, q. v.
- 19 Bilaminar blastocyst No. 55 (19), showing (at right) a mass of cells at lower pole sixty-sixth section of egg (total 139); twentieth-fourth section of embryonic area (total 77); Bouin.
- 20 Bilaminar blastocyst No. 189' (10) approaching time of mesoderm formation; 130th section of egg (total 282); ninety-third section of embryonic area (total 165); vesicle wall very thin; egg slightly damaged.
- 21 Egg No. 353' (6), blastocyst with about 140 mesodermal cells; 1.6 mm. in alcohol with embryonic area 1.1 mm.; ninety-second section (total 205); M. mesodermal cells, Bouin; $6\ \mu$.
- 22 Egg No. 346' (6); section taken through primitive streak; 1.5 mm. in diameter in alcohol; embryonic area 1.1 mm.; Bouin; $5\ \mu$; compare fig. 4, pl. 2.

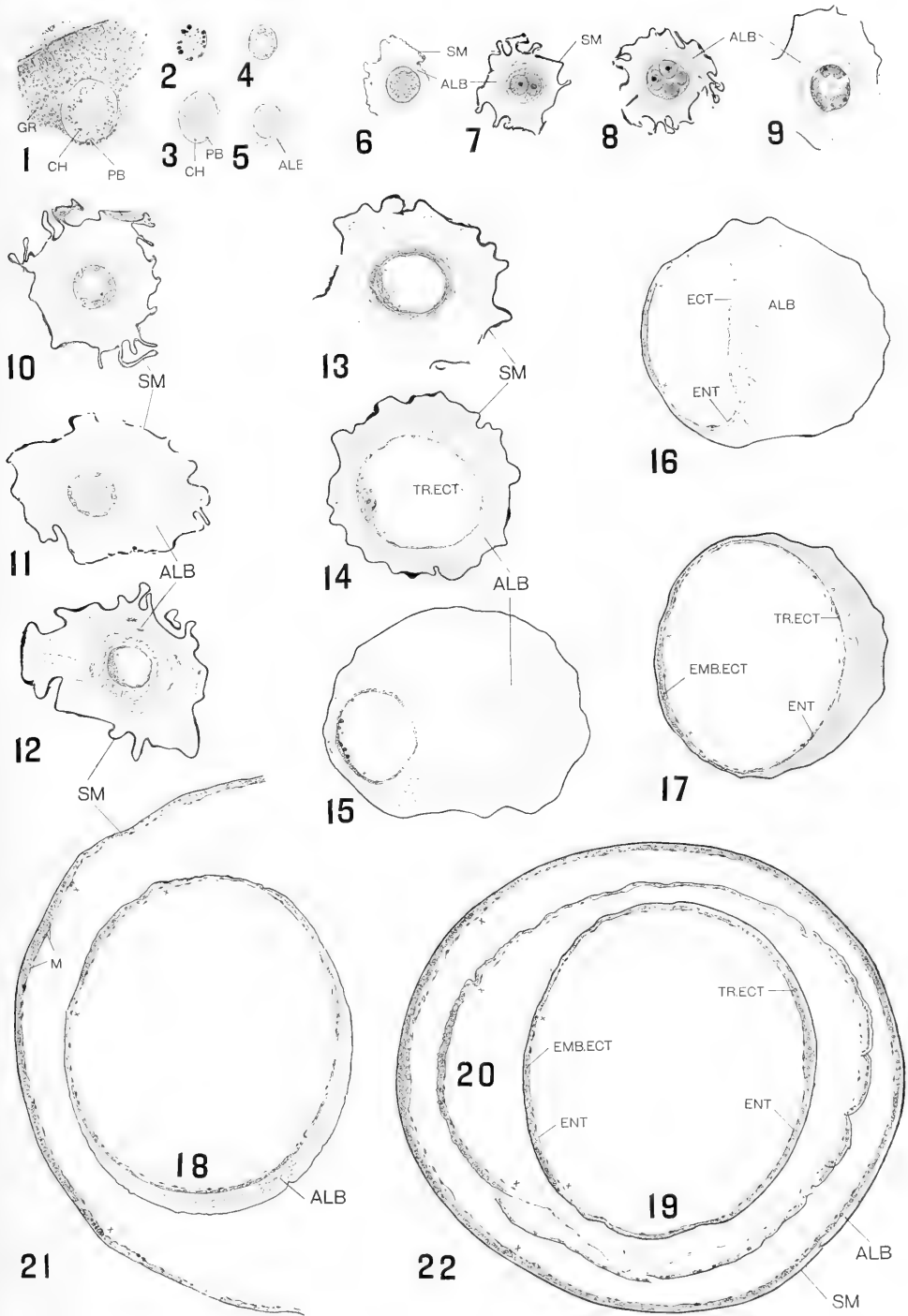


PLATE 14

EXPLANATION OF FIGURES

Maturation and fertilization.

- 1 Large ovarian egg with discus proligerus, from No. 21; 5μ . $\times 200$.
- 2 Ninth of 23 sections through tubal ovum No. 307 (1); polar body is in nineteenth section; egg is surrounded with thin albumen layer; Bouin; 5μ . $\times 200$.
- 3 Portion of fig. 2; 7 chromosomes are seen; 5μ . $\times 500$.
- 4 Tenth section of same egg; 5 chromosomes; zona pellucida is a beaded line with darkly staining granules; 5μ .
- 5 and 6 Eleventh section (total 20 sections) through ovum No. 307 (3); the second maturation spindle has 12 chromosomes; Bouin; 5μ . $\times 200$ and $\times 500$, respectively.
- 7 Sketch of ovum No. 56 (11), from total preparation drawn with focus on middle of egg; chromosomes and polar body; Bouin. $\times 200$.
- 8 Sixth section through ovum No. 76 (1); total 23 sections; 6 chromosomes of this section are in ovum and one in polar body; Bouin; 5μ . $\times 200$.
- 9 and 10 Second maturation spindle of egg No. 307 (2); tenth and eleventh sections (total 23 sections); 12 chromosomes; Bouin; 5μ . $\times 500$.
- 11 Portion of ninth section of ovum No. 76 (8); total 15 sections; 12 chromosomes in homogeneous granular area; little albumen at left; Hill's fluid. $\times 500$.
- 12 Ovum No. 313 (2); eleventh section (total 20); marginal granular zone limited within by reticulated region; oil globules of medium size; a little albumen at left; Hill's fluid; 5μ . $\times 200$.
- 13 Ovum 76 (6); composite of fifth and sixth sections (total 21); polar body and short spindle with 7 chromosomes 5μ ; Bouin. $\times 2500$.
- 14 Ovum No. 56 (6); portion of fourth section (total 17); there are 12 chromosomes; Bouin. $\times 500$.
- 15, 16, and 17 Ovum No. 76 (4); 2nd, 3rd and 4th sections tangentially (total 25 sections); polar body and equatorial plate of maturation spindle; marginal granular zone, vacuoles and oil globules; Hill's fluid; 5μ . $\times 500$.
- 18 Ovum No. 313 (5) from same litter as fig. 12; 5th section (total 20); large fat globules; albumen layer thick (compare fig. 1, pl. 3).
- 19 Young unfertilized uterine ovum, No. 287 (5); 9th section (total 16); concentric lamellae of albumen; compare fig. 12; Hill's fluid; 5μ . $\times 200$.
- 20 Ovum No. 203 (1) with pronuclei; 10th section (total 20); 5μ . $\times 200$.
- 21 Ovum No. 52 (3); composite of sections 12 to 15 (total 20) taken obliquely through first cleavage spindle; a little yolk has been extruded (Y); Bouin; 5μ . $\times 200$.

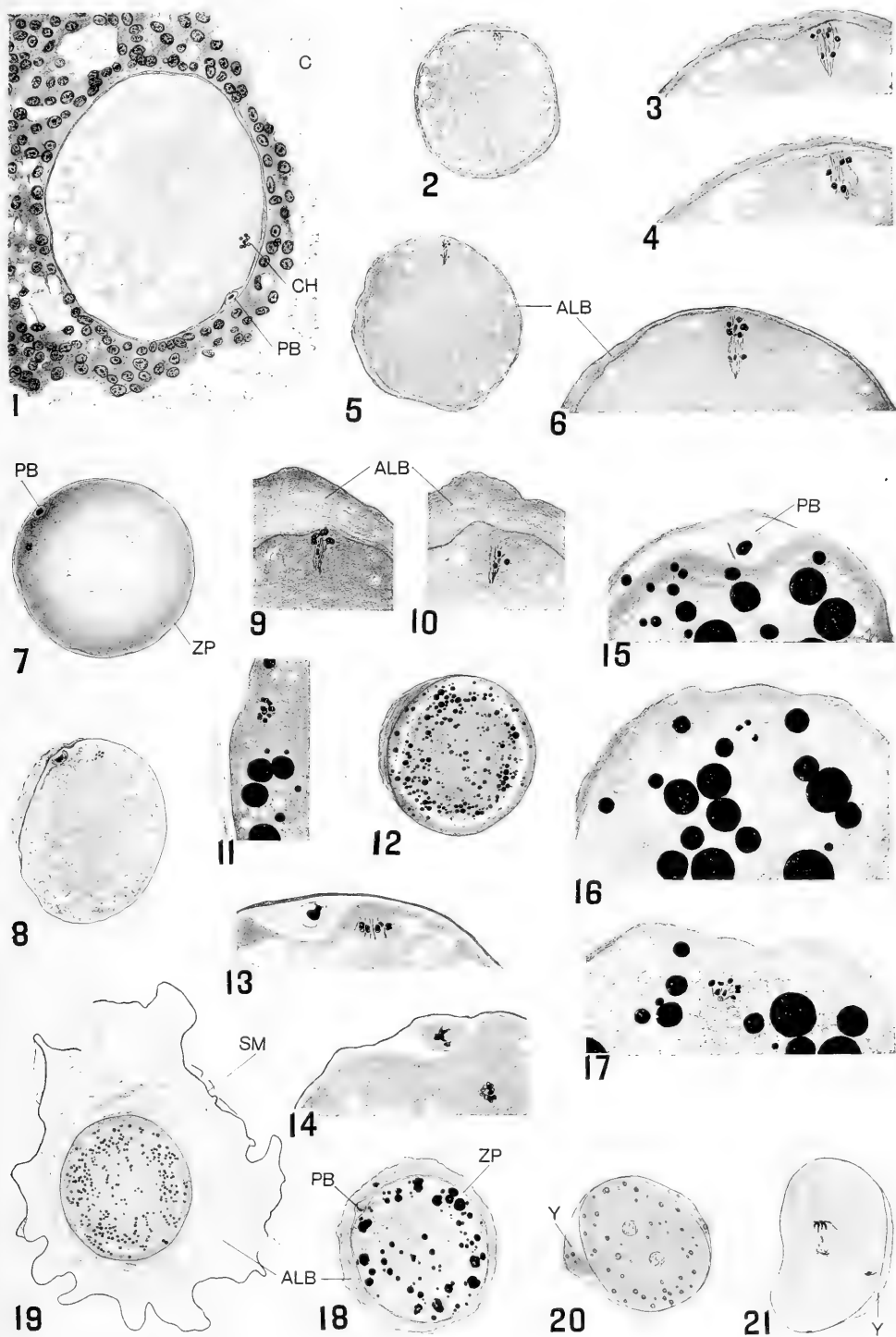


PLATE 15

EXPLANATION OF FIGURES

Cleavage stages; all magnifications $\times 200$.

1 2-celled ovum No. 203 (8), 11th section (total 20); compare figs. E and F, text fig. 4; Hill's fluid; $5\ \mu$.

2 2-celled ovum No. 306 (1); 9th section (total 23); compare figs. A and B, text fig. 4; Hill's fluid; $5\ \mu$.

3 3-celled ovum No. 173 (8); 10th section (total 21); compare fig. L, text 4; aceto-osmic-bichromate; $5\ \mu$.

4 3-celled ovum No. 306 (3); 11th of 22 sections; compare K, text fig. 4; Hill's fluid; $5\ \mu$.

5 4-celled ovum No. 293 (2), with two blastomeres in mitosis; drawn from clay model; total 18 sections, Bouin; $5\ \mu$; compare fig. 1, pl. 2.

6 4-celled ovum No. 293 (4), with two cells in mitosis; drawn from clay model; Bouin; 18 sections; $5\ \mu$; compare fig. 1, pl. 2.

7 4-celled ovum No. 203 (7); 10th section (total 20); see text; Hill's fluid; $5\ \mu$.

8 4-celled ovum No. 83 (7); 11th section (total 18); blastomeres cut as in preceding; one polar body; Bouin; $5\ \mu$.

9 and 10 5th and 9th sections through ovum No. 17' (7) one of 43 very small eggs from one ovary; total 11 sections.

11 and 12 7th and 14th sections (total 19) through ovum No. 83 (8), with portions of shell membrane and albumen; two polar bodies; Bouin; $5\ \mu$.

13 4-celled ovum No. 299 (7), also shown reconstructed in *o*, text fig. 4; 9th section (total 20); trichloroacetic; $5\ \mu$.

14 4-celled ovum No. 299 (5), of which sections 9 and 14 are shown in figs. 6 and 7, pl. 3; 13th section (total 22); Hill's fluid; $5\ \mu$.

15 6-celled ovum No. 85 (5); 7th section (total 16); blastocyst formation already anticipated; Bouin.

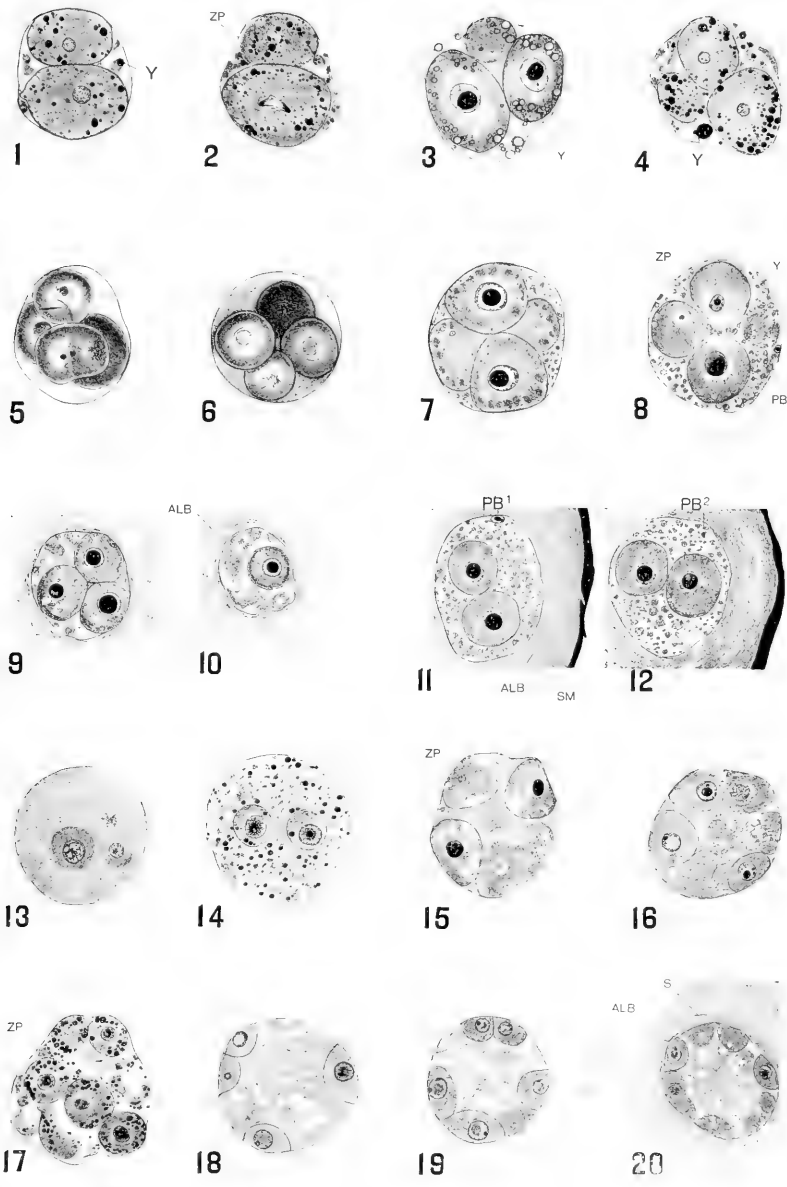
16 15-celled ovum No. 337 (1); 8th section (total 19); one-half strength Bouin; $5\ \mu$.

17 16-celled ovum No. 85 (12); 8th section (total 21); blastomeres still rounded; one misplaced cell; Hill's fluid; $5\ \mu$.

18 17-celled ovum No. 336 (1); 9th section (total 18); Bouin; $5\ \mu$.

19 26-celled ovum No. 336 (2); 11th section (total 17); Bouin; $5\ \mu$.

20 28-celled ovum No. 342 (1); 8th section (total 18); half-strength Bouin; $5\ \mu$.



EXPLANATION OF FIGURES

The formation of entoderm initiated. All magnifications are $\times 200$, except figs. 5 to 10 which are $\times 500$.

1 Completed blastocyst No. 191 (5); 34 cells; 9th section (total 24); Bouin; 5 μ .

2 Large blastocyst No. 50 (7); 70 cells, but no entoderm; 11th section (total 23); Hill's fluid.

3 Half-normal blastocyst No. 88 (18); 8th section (total 19); Hill's fluid; 5 μ .

4 Half-normal ovum No. 344 (12); 6th section (total 16); half-strength Bouin; 5 μ .

5 Portion of 6th section (total 17) through ovum No. 50 (8) showing entoderm mother cell *ENT*A; 63 cells including 2 entoderm mother cells in wall; Hill's fluid; compare fig. 11, pl. 13.

6 Portion of 8th section (total 22) of ovum No. 298 (5), showing entoderm mother cell leaving its place in blast. wall; 126 cells, of which 8 are free entoderm mother cells and several are in process of formation; Bouin, 5 μ ; cf. fig. 7 pl. 2.

7 Portion of 6th section (total 22) of ovum No. 88 (9); 106 cells of which 11 are free entoderm mother cells; Hill's fluid; 5 μ .

8 and 9 Portions of the 10th and 12th sections (total 18) through ovum No. 88 (21); 70 cells, including 10 more or less detached entoderm mother cells; Hill's fluid.

10 Greater part of 4th section (total 15) through ovum No. 88 (23); 57 cells including the two detached entoderm mother cells here shown; Bouin.

11 Blastocyst No. 88 (16), having 82 cells; 6 of the 10 entoderm mother cells are here shown; 11th section (total 20); section 15, fig. 4, pl. 7; Bouin, 5 μ .

12 Ovum No. 83 (5), containing 53 cells, including the one large binucleated entoderm mother cell (?) here shown; 9th section (total 20).

13 Ovum No. 356 (3), most retarded member of litter No. 356; about 100 cells; 12th section (total 20); Bouin; 5 μ .

14 Ovum No. 344 (4); small blastocyst with numerous entoderm mother cells; 8th section (total 18); Hill's fluid; 5 μ .

15 Longitudinal section of ovum No. 344 (11), showing definite polar differentiation; typical entoderm mother cells; 7th section (total 19); half-strength Bouin; 164 cells:

Embryonic ent-ectoderm.....	71 cells of which 7 are in mitosis
Trophoblastic ectoderm.....	70 cells of which 8 are in mitosis
Entoderm.....	23 cells of which 3 are in mitosis

16 and 17 The 7th and the 16th sections (total 22) taken horizontally through ovum No. 344 (14), slightly more advanced than preceding; fig. 16, through embryonic area; fig. 17, through trophoblastic area; half-strength Bouin; 5 μ ; 193 cells:

Embryonic ent-ectoderm.....	76 cells, 8 in mitosis
Trophoblastic ectoderm.....	98 cells, 13 in mitosis
Entoderm.....	19 cells, 1 in mitosis

18 to 22 Reconstructions from blastocysts to show the polar distribution of entoderm mother cells. Fig. 18, ovum No. 88 (17), 103 cells, of which 6 are entoderm mother cells; section indicated by parallel lines is shown in fig. 2, pl. 7. Fig. 19, ovum No. 83 (1), 111 cells, of which 4 are free entoderm mother cells. Fig. 20, ovum No. 298 (3), 124 cells, of which 4 are entoderm mother cells. Fig. 21, ovum No. 88 (7), 87 cells, including 5 entoderm mother cells; the section indicated by lines is shown in fig. 3, pl. 6. Fig. 22, ovum No. 88 (11), 103 cells, of which 9 are more or less free entoderm mother cells and 7 of these are in mitosis.

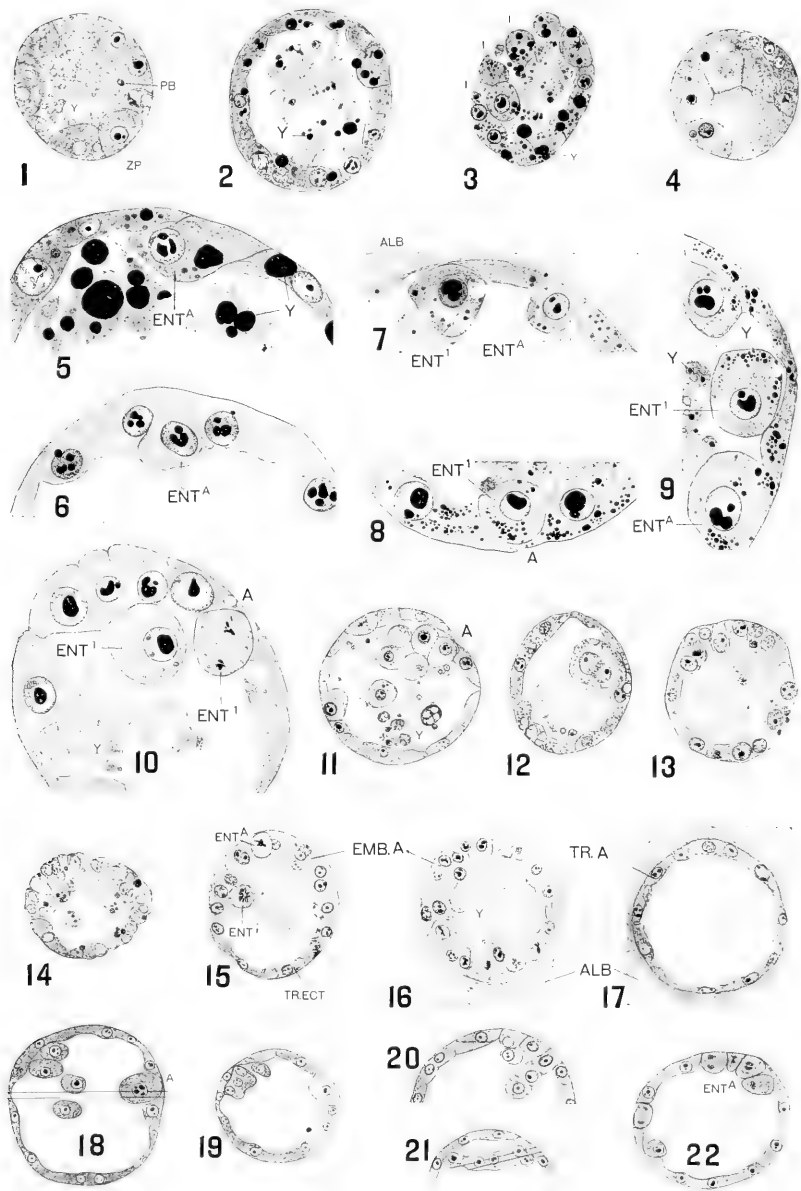


PLATE 17

EXPLANATION OF FIGURES

The formation of entoderm (concluded).

1 The 7th section longitudinally through ovum No. 144' (1); *ENT*¹, dividing entoderm mother cell; 16 sections in series; overfixed in Carnoy. $\times 200$.

2 The 14th section longitudinally through ovum No. 144' (8); 19 sections in series; Carnoy. $\times 200$.

3 Section taken tangentially through embryonic area of ovum No. 144' (10); 14th section (total 19); Carnoy. $\times 200$.

4 and 5 Sections 9 and 11 (total 25) cut longitudinally through blastocyst No. 356 (11); Hill's fluid; $5\ \mu$; *ENT*³, primitive entoderm cell tending to flatten out; *ENT*², row of entoderm mother cells similar to those in fig. 3, pl. 6. $\times 200$.

6 and 7 Details of ovum No. 356 (4) shown in fig. 12, pl. 13. Fig. 6, 10th section (total 25), $\times 200$; fig. 7, 16th section, $\times 500$, with spermatozoa in albumen layer; mitosis in embryonic entectoderm; Bouin; $6\ \mu$; 283 cells:

Embryonic ent-ectoderm.....	101 cells, in mitosis 3
Trophoblastic ectoderm.....	140 cells, in mitosis 3
Entoderm.....	42 cells, in mitosis 2

8 and 9 Portions of sections 10 and 17 (total 29) longitudinally through ovum No. 356 (9), shown whole in fig. 1, pl. 6; Flemming; $5\ \mu$. $\times 500$.

10 and 11 Sections 18 and 13, respectively (total 21), longitudinally through ovum No. 356 (5), section 12 of which is shown in fig. 3, pl. 9; mitoses in ent-ectoderm; Bouin; $6\ \mu$; 249 cells:

Embryonic ent-ectoderm.....	75 cells, in mitosis 14
Trophoblastic ectoderm.....	126 cells, in mitosis 11
Entoderm.....	48 cells, in mitosis 4

12 Longitudinal section of egg No. 349 (2) like the one shown in living stage is fig. 3, pl. 8; 31st section through vesicle (total 43); 16th section through embryonic area (total 28); Bouin; $5\ \mu$. $\times 200$.

13 Longitudinal section through ovum No. 194' (4); 7th section through embryonic area, (total 20); Hill's fluid; $7\ \mu$. $\times 200$.

14 The 9th of a total of 13 sections through the embryonic area of ovum No. 194' (8). $\times 500$.

15 The 12th of a total of 20 sections through the embryonic area of blastocyst No. 194' (6); 38 sections through vesicle; $7\ \mu$. $\times 500$.

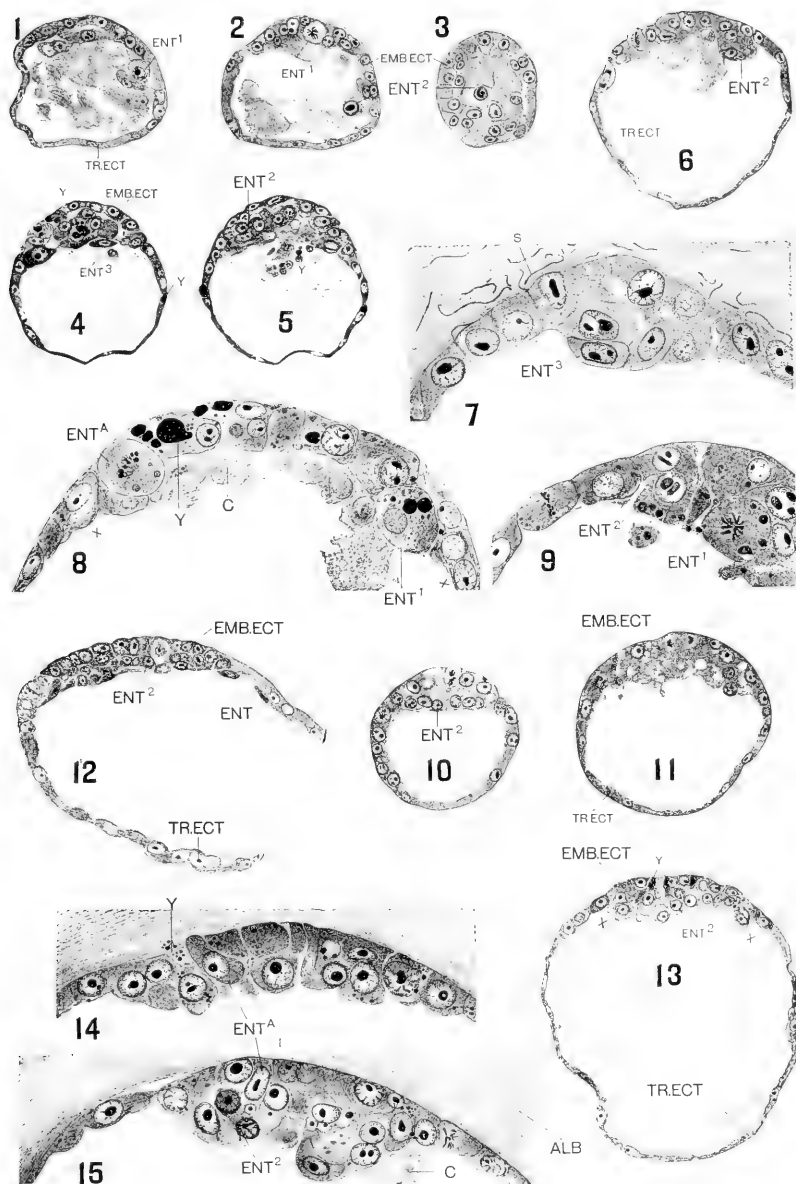


PLATE 18

EXPLANATION OF FIGURES

Stages from the spreading of the entoderm to the just completed bilaminar blastocyst. Whole sections (figs. 5A, 6A, 7A) $\times 50$; vesicles only $\times 200$; *ENT*, limits of distribution attained by the entoderm; *ENT*^c, undifferentiated primitive entoderm not yet spread.

1 Blastocyst No. 43 (7); 10th section through embryonic area; Bouin.

2 Blastocyst No. 352 (12); 42nd section through egg (total 98), 33d, section through vesicle (total 80), and 18th section through embryonic area (total 50); half-strength Bouin; $5\ \mu$; compare fig. 1, pl. 9.

3 Blastocyst No. 40 (1); 15th section through embryonic area (total 30); Hill's fluid.

4 Blastocyst No. 40 (2); the 19th section through embryonic area (total 36); Carnoy.

5A and 5 Blastocyst No. 347 (2); earliest stage of the completed bilaminar blastocyst; 22nd section through vesicle (total 57); Bouin; $7\ \mu$; compare fig. 5, pl. 9.

6A and 6 Blastocyst No. 339 (3). Fig. 6A, 66th section of vesicle (total 94) and 37th section of embryonic area (total 51); fig. 6, 68th section of vesicle; Bouin; $5\ \mu$; compare fig. 6, pl. 9, and fig. 2, pl. 6.

7A Egg No. 347 (1); 52nd section through egg (total 127); 46th section through vesicle (total 103); entoderm spread to equator.

7 Blastocyst No. 347 (4); nearly the same stage as fig. 7A; 32nd section through vesicle (total 90); Bouin; $7\ \mu$.

8 Blastocyst No. 175' (2); 9th section through embryonic area (total 25) and 30th section through vesicle (total 56); aceto-osmic-bichromate; $6\ \mu$.

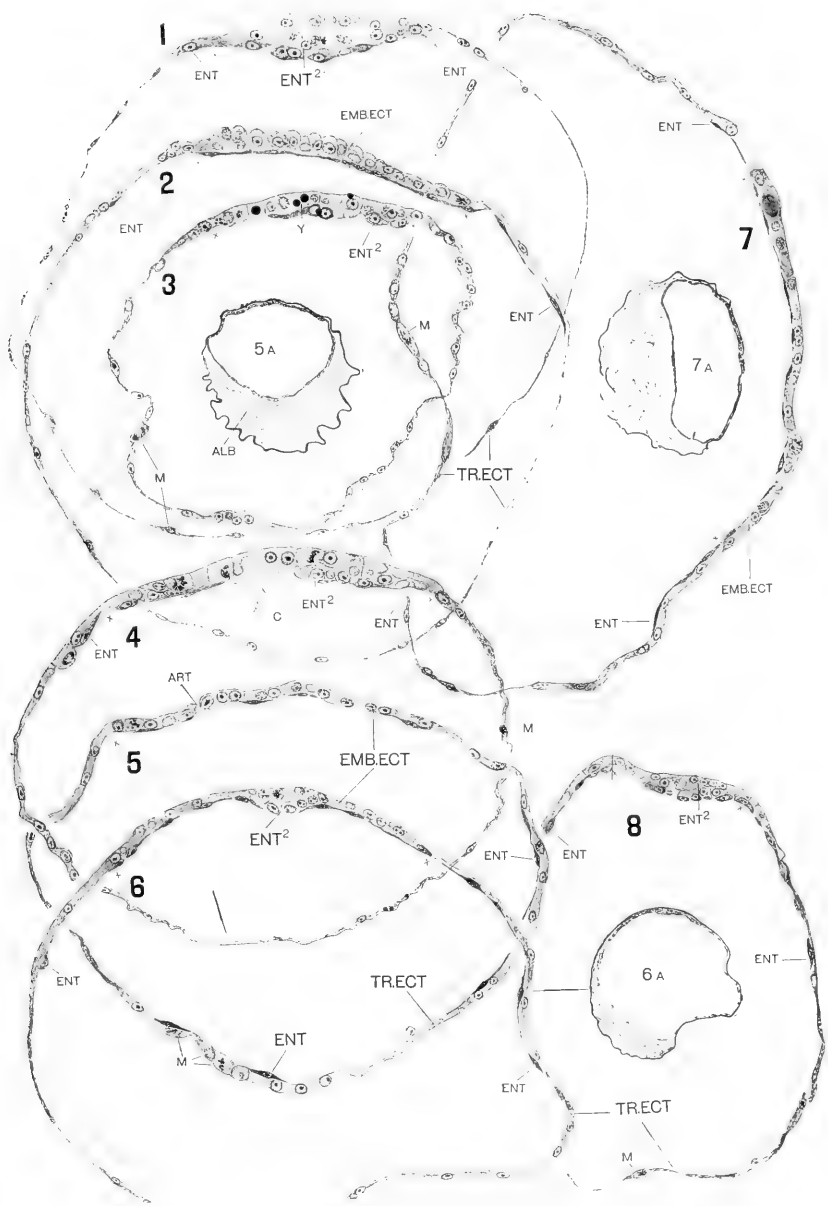


PLATE 19

EXPLANATION OF FIGURES

Partially completed and just completed bilaminar blastocysts.

1 Longitudinal section through ovum No. 205 (4); 41st section (total 93); Bouin; 7 μ . $\times 50$.

2 Surface view of trophoblastic area of an egg from the litter No. 205; entoderm shaded; ectodermal nuclei unshaded. $\times 500$.

3 Detail of embryonic area of blastocyst No. 347 (4), shown in fig. 7, pl. 18. $\times 500$.

4 Detail of section through embryonic area of ovum No. 352' (10), shown in fig. 4, pl. 10; $\times 500$.

5 and 5A Entire section, $\times 50$, and vesicle only, $\times 200$, through the middle of ovum No. 339 (4), photographed in toto in fig. 2, pl. 6; note swollen cells; Bouin; 5 μ .

6 Embryonic area only of similar egg No. 339 (2); Bouin; 5 μ . $\times 200$.

7 Blastocyst No. 175' (9), with very attenuated, mostly unilaminar wall; 52nd section through vesicle (total 89); aceto-osmic-bichromate (?); 5 μ . $\times 50$.

7a Embryonic area only of same egg. $\times 200$.

8 and 8A Entire section, $\times 50$, and embryonic area (XX), $\times 200$, of ovum No. 347 (5); entoderm has not yet reached equator; 30th section of vesicle (total 119) and 18th section through area (total 29); Flemming; 5 μ .

9 The embryonic area of ovum No. 205 (6); 49th section of blastocyst (total 79); Bouin; 5 μ ; compare fig. 1.

10 Just completed bilaminar blastocyst No. 208 (1); 56th section through vesicle (total 117); Bouin. $\times 50$.

10A and 10B Details of embryonic area and trophoblastic area of same egg. $\times 200$.

11 Portion of surface view of ovum No. 205 (7); XX, junctional line; only the entoderm is shaded; ectodermal nuclei unshaded circles. $\times 500$.

12 Surface view at junctional line (XX) of ovum No. 205 (9); entire ectoderm shaded; embryonic nuclei very dark, trophoblastic nuclei very light; entodermal nuclei intermediate in tone.

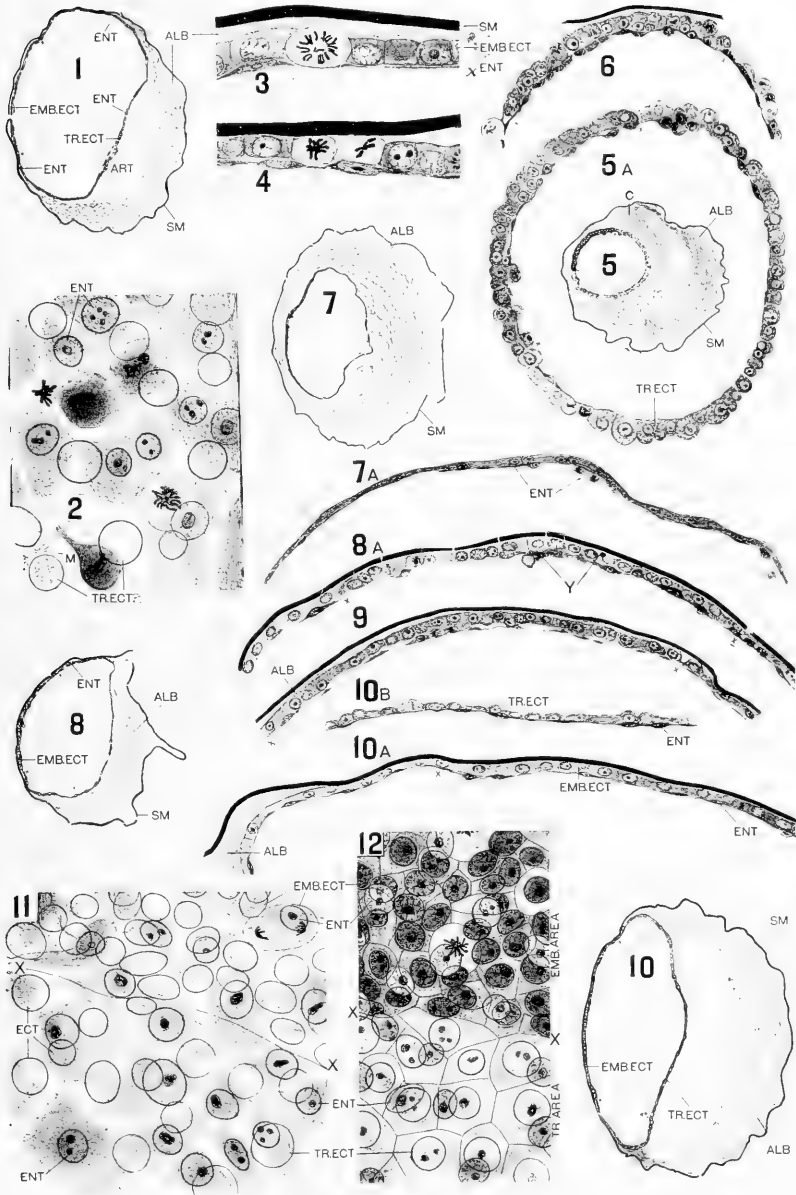


PLATE 20

EXPLANATION OF FIGURES

Completed bilaminar blastocyst.

1 Section of embryonic area (XX) of blastocyst No. 82 (13), nearly like fig. 1, pl. 21; 54th section of egg (total 100) and 38th section of embryonic area (total 58); Bouin. $\times 200$.

1A Detail of same. $\times 500$.

2 Blastocyst No. 306' (2) shown in fig. 17, pl. 13; 0.77 mm. in diameter in alcohol; 18th section of embryonic area (total 49) and 63d through vesicle (total 118); compare fig. 7, pl. 10; Hill's fluid; $5\ \mu$. $\times 200$.

2A Detail of same near junctional line (X). $\times 500$.

3 Ovum No. 285' (1); 72nd section through blastocyst (total 107); Bouin; $5\ \mu$; the portion of vesicle marked by dotted line was dissected off before embedding and was stained and mounted in toto (fig. 3B). $\times 50$.

3A Same blastocyst. $\times 200$.

3B Surface view from point A, fig. 3; junctional line XX; ectoderm lightly shaded; entodermal nuclei darkly shaded. $\times 500$.

4. The 92nd section (total 140) through blastocyst No. 43 (10); two degenerating cells at A; Hill's fluid. $\times 50$.

4A The embryonic area (XX) of same. $\times 200$.

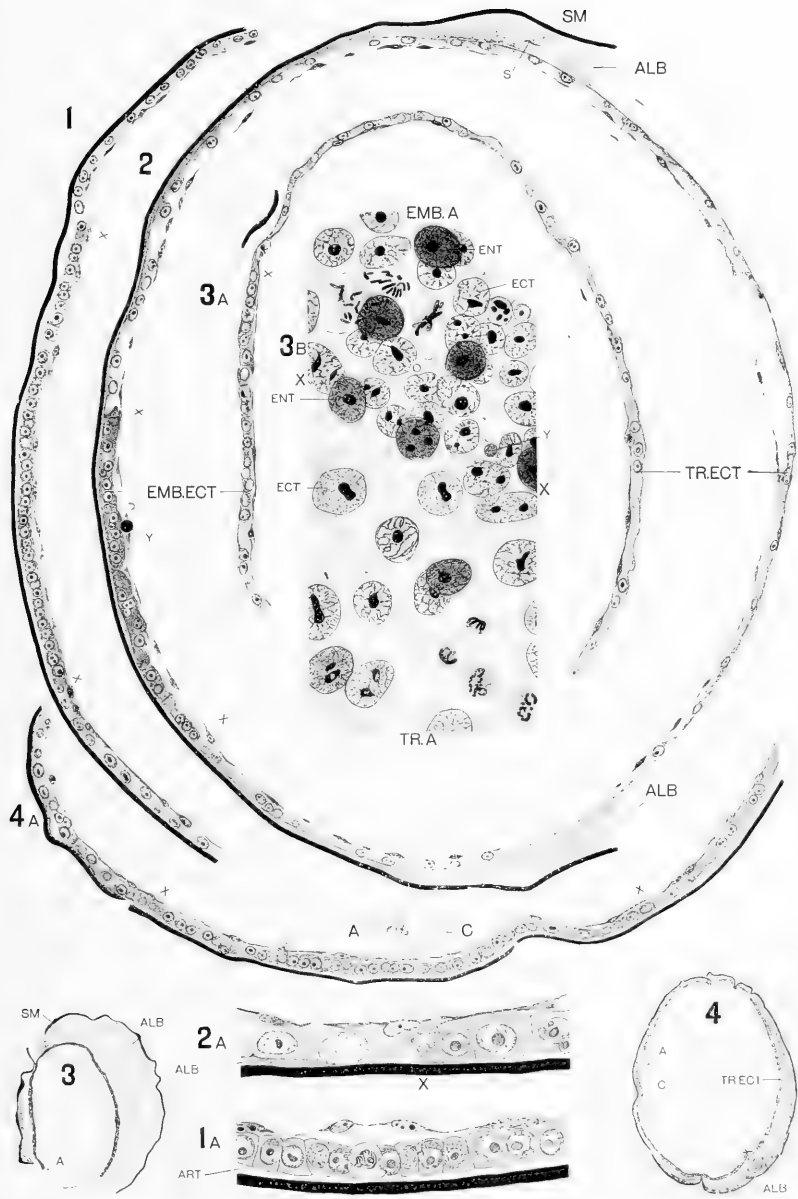


PLATE 21

EXPLANATION OF FIGURES

The 1-mm. bilaminar blastocyst.

1 Egg No. 306' (3); 0.85 mm. in diameter in alcohol; 44th section of embryonic area (total 70) and the 84th section of vesicle (total 135); Bouin; 5 μ . \times 50.

1A Embryonic area (XX) of same section. \times 200.

2 Egg No. 55 (20); 0.87 mm. in diameter in alcohol; 57th section of vesicle (total 121) and 33d section of embryonic area (total 77); O, pocket in trophoblastic ectoderm; Flemming; 6 μ . \times 50.

2A Embryonic area (XX) of same section; at A, unusual crowding of ectoderm. \times 200.

3 Egg No. 339' (3); 0.85 mm. in alcohol; 64th section of vesicle (total 118) and 45th section of embryonic area (total 71); O, pocket in ectoderm; half-strength Bouin; 6 μ . \times 50.

4 Embryonic area (XX) of egg No. 189 (6), shown in fig. 18, pl. 13; 74th section of embryonic area (total 94); 1.02 mm. in alcohol; Hill's fluid; 5 μ . \times 200.

5 Embryonic area (XX) of egg No. 343 (4), about 1.0 in alcohol (compare fig. 5, pl. 2, and fig. 9 below); 99th section of vesicle (total 189) and 67th of embryonic area (total 126); Bouin; 5 μ . \times 200.

6 A portion of trophoblastic area of a 1.0 blastocyst No. 55 (6), showing remnant of albumen; compare fig. 10, pl. 22; Hill's fluid. \times 200.

7 and 7A Sections through embryonic and trophoblastic areas of ovum No. 285' (6); 65th section of vesicle (total 119) and 26th through embryonic area (total 65); Hill's fluid; 6 μ . \times 200.

8 Embryonic area (XX) of ovum No. 352 (7); 35th section of egg (total 80); 24th section of vesicle (total 49) and 13th section of embryonic area (total 21); in alcohol egg measured 0.585 mm. through shell membrane and 0.325×0.370 through vesicle; 6 μ ; Bouin. \times 200.

9 Surface view of a typical 1 mm. blastocyst, showing embryonic area; compare fig. 2, pl. 11, and fig. 3, pl. 10. \times 16.

10. Half-normal blastocyst No. 314 (3); Bouin; 5 μ . \times 200.

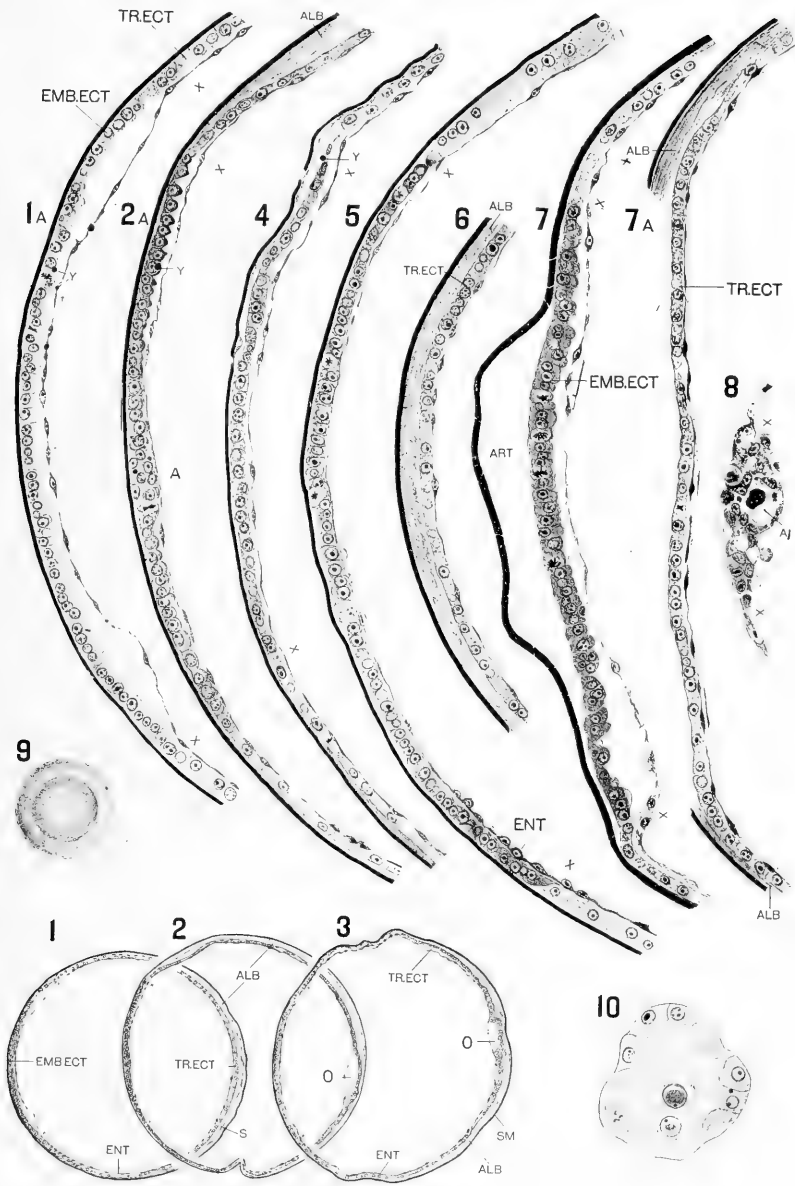


PLATE 22

EXPLANATION OF FIGURES

Advanced bilaminar blastocysts, to the beginning of mesoderm proliferation. Figs. 2, 4, and 9 represent eggs only a few minutes removed from the first appearance of mesoderm.

1 Egg No. 193' (4), similar to fig. 9 below; a 92nd section of egg (total 184) and 55th section of embryonic area (total 87); Hill's fluid; 7 μ . \times 50.

2. Egg No. 343' (2), one of the five shown in fig. 6, pl. 2; 1.5 mm. in alcohol; embryonic area, 0.87 mm.; 130th section of vesicle (total 259) and 89th section through embryonic area (total 161); Bouin; 5 μ . \times 50.

3 Egg. No. 353 (4); several hours preceding first appearance of mesoderm; diameter 1.22 mm. in alcohol; 91st section of vesicle (total 135) and 53rd section of embryonic area (total 85); Flemming; 6 μ . \times 50.

3A and 3B Details of trophoblastic and embryonic areas, respectively, of same section. \times 200.

3C A detail of fig. 3B. \times 500.

4 and 4A Egg No. 189' (1); 118th section of vesicle (total 200) and 62nd section of embryonic area (total 116); aceto-osmic-bichromate. \times 50 and \times 200.

5 Embryonic area (XX) of egg No. 347' (1); 1.1 mm. in alcohol; 74th section of vesicle (total 126) and 55th section of embryonic area (total 82); Flemming; 7 μ . \times 200.

6 Thick embryonic area (XX) of egg No. 360 (4); 71st section of vesicle (total 176) and 41st of embryonic area (total 130); *T*, thinning near middle; Hill's fluid; 5 μ . \times 200.

7 The 79th section through embryonic area (total 172) of egg No. 189' (9). \times 200.

8 Large embryonic area of egg No. 189' (4); 157th section of egg (total 311) and 59th section of area (total 169); Hill's fluid; 5 μ . \times 200.

9 Drawing made from photograph of an egg in litter No. 193'; shows central light field in embryonic area; compare figs. 1 and 2, pl. 10. \times 16.

9A Section of ovum No. 193' (2); 61st section of embryonic area (total 160). \times 200.

9B and 9C Details of portions of fig. 9A. \times 500.

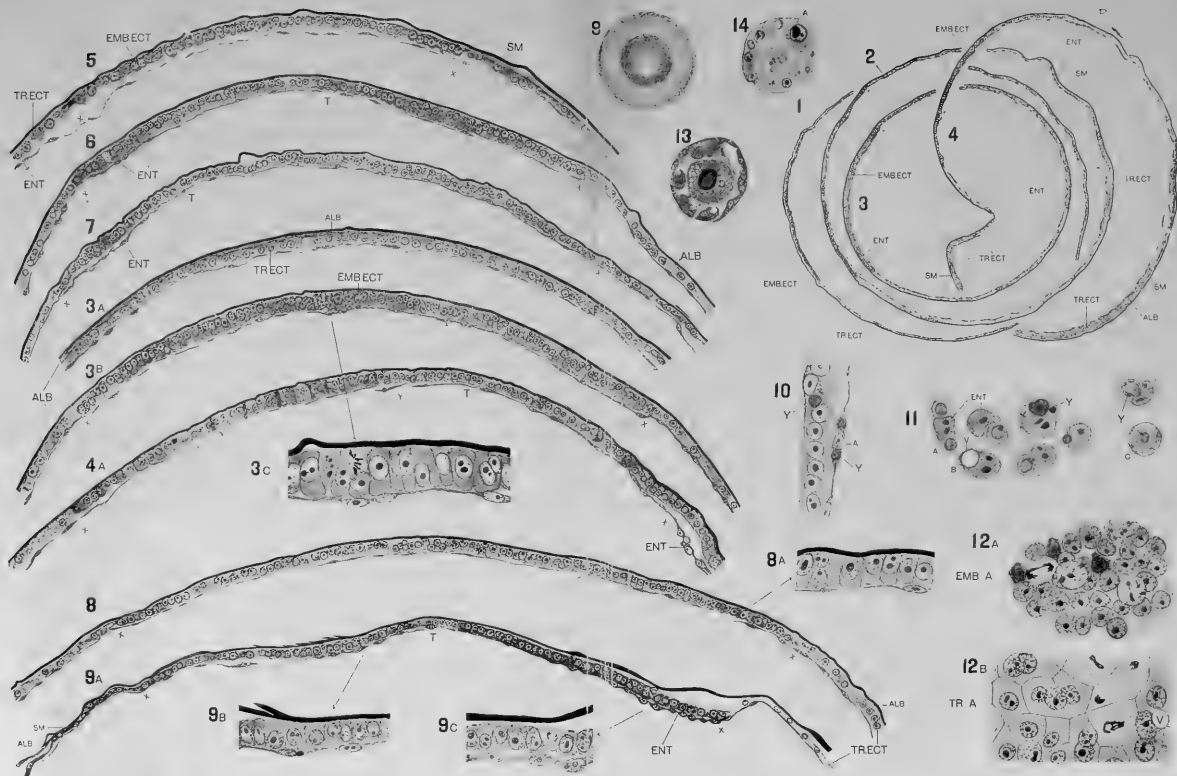
10 Portion of embryonic area of 1-mm. blastocyst No. 55 (6), showing yolk granules (*Y*) in ectoderm, and entoderm; compare group A with A, fig. 11.

11 Surface view of some entoderm cells from below the embryonic area of egg No. 189' (12), showing reaction of cells to yolk remnants (*Y*).

12A and 12B Surface views, from within, of embryonic and trophoblastic areas of egg No. 189' (11); entodermal nuclei (mostly the larger) are seen above the entoderm; embryonic area measures 0.96 mm.; aceto-osmic-bichromate. \times 500.

13 Defective blastocyst No. 88 (6) with large included blastomere; Hill's fluid; 5 μ . \times 200.

14 Half-normal blastocyst No. 356 (2); Bouin; 5 μ . \times 200.



Resumido por el autor, Edward Phelps Allis, jr.

Los labios y orificios nasales en los peces gnatostomos.

En los vertebrados existen tres clases de labios funcionales, primarios, secundarios y terciarios. Los labios primarios están colocados en posición inmediatamente aboral al arco cuadrado-mandibular; son funcionales en los Ciclóstomos, en la porción media de la hendidura bucal de los Plagiostomos y probablemente también en los Condrósteos; están situados siempre en posición oral respecto a los orificios nasales. Los labios secundarios están formados por un pliegue del dermis externo, el cual primitivamente cruza el ángulo lateral de la boca, pero más tarde se extiende hasta que encuentra el del lado opuesto con el que se une en forma de sínfisis. La posición de estos labios es aboral con relación a los primarios y están representados en los embriones por los procesos maxilar, mandibular y fronto-nasal; la banda de dermis externa situada entre ellos y los labios primarios se incluye secundariamente en la cavidad bucal. Estos labios son funcionales en las porciones laterales de la hendidura bucal de los Plagiostomos y en toda la longitud de dicha hendidura en los Teleostomos, Anfibios y Amniotos; están colocados en posición inmediatamente aboral al arco maxilo-dentario, y el labio superior en posición oral con relación a los dos orificios nasales (Teleostomos, la mayor parte de los Plagiostomos) o entre dichos orificios (Heterodontus, Anfibios, Amniotos). Los labios terciarios están situados en posición aboral con relación a los secundarios y orificios nasales y se encuentran solamente en la mandíbula de los Dipnoos. Cuando la cresta del pliegue del labio superior secundario encuentra el orificio oro-nasal se interrumpe y de este modo se origina un surco naso-bucal. En los Holocéfalos un conducto nasal secundario, colocado entre ambos orificios nasales, se desarrolla exteriormente al puente nasal de los peces, entre éste y un pliegue naso-labial que le recubre, y este conducto se transforma, aparentemente, en el conducto nasal definitivo de los Anfibios.

THE LIPS AND THE NASAL APERTURES IN THE GNATHOSTOME FISHES

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SIXTEEN FIGURES

His ('92 b), in a work largely embryological and embracing all classes of the Craniata, came to the conclusion that there were four kinds of lips in these vertebrates:

1. Die Lippe der höheren Wirbelthiere und der Amphibien, welche durch Verschmelzung des mittleren Stirnfortsatzes mit den Oberkieferfortsätzen entsteht und die vor den primären Choanen liegt.

2. Die Lippe der Knochenfische, an deren Bildung der mittlere Stirnfortsatz zwar Theil nimmt, aber deren Ort unterhalb der primären Choanen fällt.

3. Die Oberlippe der Selachier, welche ohne Betheiligung des mittleren Stirnfortsatzes unterhalb der Riechgrube entsteht. Wenn wir die erste Form als 'Gesichtslippe' bezeichnen, so können wir die Formen 2 und 3 vielleicht 'Gaumenlippen' nennen.

Eine vierte Form ist die 'Rauchenlippe', welche wir weiter unten bei Besprechung der Petromyzontenschnauze werden kennen lernen; sie hat ihren Ausgangspunkt hinter dem Eingang in die Rathke'sche Tasche.

Keibel ('93), in a work relating to this same subject, quotes these four paragraphs from His's work and then says that he considers "die Hauptfrage durch His gelöst," but that he differs from him in regard to certain points, one of which is that the upper lips of the Teleostei and Selachii develop in a strictly similar manner and are accordingly homologous instead of non-homologous. In a later work Keibel ('06) reconsiders this subject, at somewhat greater length, and reasserts his earlier conclusions regarding it.

My work, limited almost exclusively to the adults of the gnathostome fishes, leads me to quite different conclusions. The conditions found in these fishes will be first described, and then brief comparison made with the conditions in higher vertebrates.

PLAGIOSTOMI

In *Chlamydoselachus* the lips are much thicker at the angle of the gape of the mouth than in their more anterior portions, the angle of the gape thus being a relatively long line. The inner end of this line forms the functional angle of the gape when the mouth is widely opened, and the outer end of the line the functional angle when the mouth is closed, and from this outer angle the outer edge of each lip converges toward the inner edge until the lips attain their normal thickness. This is readily seen in the accompanying figures 1 and 2, as also in two similar figures given by Garman in 1885, and it is there also seen that what is actually a portion of the external surface of the head when the mouth is widely opened, becomes enclosed between the lips when the mouth is closed.

The cause of this thickening of the lips at the angle of the gape was, in the first place, the inevitable formation of a fold in the loose dermis at that angle, such a fold being well shown in Müller and Henle's (1841) figure of *Pristis antiquorum*, reproduced in the accompanying figure 3, but this slight fold was later enlarged, apparently because of the pressure of the thick concave anterior edge of the *musculus adductor mandibulae*, where it passed from the upper to the lower jaw, against the internal surface of the fold. Because of this pressure, the fold was forced outward and forward, and, when the mouth was closed, bulged across the primary angle of the gape, its anterior surface being presented symphysially and internally and added to the lips at the angle of the gape. The crest of this fold then formed, when the mouth was closed, a secondary angle of the gape, which lay antero-lateral to the primary angle, and short secondary upper and lower lips ran forward from it and joined the primary lips. That portion of the external surface of the head which lay between these secondary lips and the primary ones was then first added to the lips at the angle of the gape but later incorporated in the buccal cavity by the formation of a cheek. The secondary lips, because of the manner of their formation, are not at first represented by the

crest of the fold which gives origin to them, that crest diverging more or less from the primary lips and the fold gradually spreading out upon the external surface of the head and vanishing. There is accordingly quite frequently a break in the definitive lip, particularly the upper lip, between the primary lips and the crest of the fold of the secondary lip; as seen in the accompanying figures of *Mustelus* and *Scyllium* (figs. 5, 8).

In all of the few other Plagiostomi that I have been able to examine, secondary lips are found which are strictly comparable to those above described in *Chlamydoselachus*, but the short secondary lips of the latter fish may be extended much farther forward, and there are marked variations in the upper lip due mainly to the varying relations of the nasal apertures to the upper edge of the mouth. These variations, in the few fishes I have been able to examine, will be considered in connection with the descriptions of the nasal apertures, but it may here be stated that, as a general rule, where the nasal apertures lie at a considerable distance from the upper edge of the mouth, the secondary upper lip passes between those apertures and that edge of the mouth, but when the oral nasal aperture lies near the upper edge of the mouth, the fold of the secondary upper lip is interrupted or displaced by its encounter with that aperture.

Related to the secondary angle of the gape there are, as is well known, in most, but not all of the Plagiostomi, dermal furrows, more or less developed. One of these furrows lies in the upper jaw, dorsal and internal to the one or two upper labial cartilages, and it usually turns downward, posterior and internal to the articulating hind ends of those labials with the mandibular labial, and then forward (symphysially) a short distance aboral and internal to the latter labial. A dermal flap, or fold, enclosing the articulating hind ends of the upper and lower labials, is thus formed, and it may be called the labial fold. The related furrow is indistinctly separated into two parts in all the Selachii I have examined, these two parts being confluent in their superficial portions but slightly separated from each other in their deeper portions. They are, however, apparently simply parts of a single furrow and can be called, together, the labial furrow; the two

parts, where necessary to designate them separately, being called the supralabial and the postlabial furrows. A third furrow lies in the lower jaw immediately and hence superficial to the mandibular labial. It extends posteriorly as far as the ventro-anterior end of the postlabial furrow, but ends dorsal (oral) to that furrow, not running directly into it, the two furrows being confluent superficially, but distinctly separate in their deeper portions. This third furrow may be called the supramandibular furrow and the related fold the supramandibular fold, the term mandibular being avoided because the fold and furrow both lie external and hence anterior to the mandibular labial, and the term premandibular not being used because it implies something belonging to a premandibular arch or region. The labial and supramandibular folds together, form, in the *Plagiostomi*, a single large fold which is usually but not always separated into dorsal (maxillary) and ventral (mandibular) portions by a posterior continuation of the line of the angle of the gape. Two other furrows are usually found, one in each jaw, running forward (symphysially) from the line of the angle of the gape, not far from its inner end. They are both short, and were apparently primarily simply creases in the dermis between the folds of the primary and secondary lips. The crease in the upper lip runs symphysially, diverging slightly from the line of the primary upper lip. The crease in the lower lip, in the few specimens I have examined, curves aborally and approaches the outer end of the line of the angle of the gape, thus circumscribing a small islet of dermis which lies immediately symphysial to the line of the angle of the gape and external to the primary lower lip; the islet accordingly belonging to the tissues of the secondary lip. These little furrows will hereafter be referred to as the maxillary and mandibular preangular labial creases.

In *Chlamydoselachus* these several furrows are not well developed, the supralabial and supramandibular furrows being simply creases in the dermis which do not run together posterior and internal to the hind ends of the labials. There is accordingly no postlabial furrow, and hence no labial fold, properly

so-called, in this fish. In *Mustelus* (probably *vulgaris*) and *Triakis fasciatum* I find all the furrows well developed, and they are shown in the accompanying figures of *Mustelus* (fig. 8). In *Scyllium canicula* (fig. 5) I find the supramandibular furrow and the two preangular creases well developed, but there is no postlabial or supralabial furrow. In *Raia clavata* none of these furrows are found as such, but the naso-buccal groove has probably absorbed the maxillary preangular crease, as will be later explained.

In the adults of all of the Plagiostomi the primitive single external opening of the nasal pit is more or less completely, but never completely, separated into two parts either by the well known nasal flap, which projects from one side of the primitive nasal opening and rests upon a flap seat on the other side, or by the nasal flap and seat together with two deeper-lying flaps, one on either side, which together form what I shall call the nasal valve and its valve seat. The nasal flap and nasal valve of one side of the nasal pit, and the flap-seat and valve-seat of the other, correspond to the two halves of the well known nasal bridge of the Teleostei, but these two halves of the bridge never fuse with each other in the Plagiostomi, the two nasal apertures never, in consequence, being completely separated from each other.

The two nasal apertures lie, in the adults of all of the Plagiostomi that I have examined or can find described, one lateral or antero-lateral to the other, and it is always the lateral one of the two which serves for the ingress of the current of water passing through the nasal pit and the other for its egress. The nasal groove of embryos of these fishes, as shown in figures, always runs from in front orally and mesially, as does the line of the external nasal apertures of the adult, but the line of the groove is always inclined to the axis of the body at a smaller angle than the line of the apertures. The line of the median raphe of the Schneiderian membrane, lies, in the adult, approximately in the plane of the long axis of the fenestra nasalis, and crosses the line of the external apertures at a variable angle, running, where the mouth is ventral and in the few speci-

mens I have examined, from behind mesially and more or less aborally, apparently tending to become approximately parallel to the upper edge of the mouth.

Neither the line of the external apertures of the adult nor the line of the median raphe of the Schneiderian membrane thus lies in the direction of the nasal groove of embryos, this apparently having been caused by, or being related to, a change in direction of the long axis of the external opening of the nasal pit. The descriptions and figures of embryos do not permit the several stages in this change in direction of this axis to be followed, but that there is such a change, and that it has the character of a partial rotation of the axis of the opening in the plane of that opening is evident from a comparison of the conditions shown by Berliner ('02) in embryos of *Acanthias* with those found in the adult of that fish; the central line of the opening of the nasal pit of embryos, and the line of the median raphe of the Schneiderian membrane, both lying approximately in the line of the nasal groove and hence directed from in front orally and mesially, while in the adult the long axis of the fenestra nasalis and the line of the median raphe are directed aborally and mesially, the line of the external apertures crossing this line at a considerable angle and being directed mesially. The appearance is accordingly that of the long axis of the fenestra nasalis having rotated from left to right through a considerable angle, carrying the median raphe with it and dragging, at either end, the related external aperture a certain distance from its embryonic position. The rotation of the nasal apertures is accordingly less extensive than that of the axis of the fenestra, and the passage from each aperture into the nasal capsule is, in consequence, pulled out and lengthened to a variable extent, the two passages being directed in opposite directions.

In the adult *Mustelus* (probably *vulgaris*) the long axis of the fenestra nasalis extends from in front orally and laterally, lying approximately parallel to the upper edge of the mouth and coinciding in direction with the line of the median raphe of the Schneiderian membrane (figs. 8 and 9). The line of the centers of the external nasal apertures crosses this line at a considerable an-

gle, extending from in front mesially and slightly orally, the antero-lateral aperture leading orally into the postero-lateral end of the fenestra nasalis and the postero-mesial aperture leading aborally into its antero-mesial end. The long axis of the fenestra nasalis and the median raphe have accordingly here both swung partly round a circle, dragging the external apertures after them, as in *Acanthias*.

The postero-mesial two-fifths of the edge of the fenestra nasalis of this fish is of membrane, the remaining three-fifths of cartilage. The ala nasalis (Nasenflügelknorpel) encircles about four fifths of the fenestra and fits against the inner edge of its cartilaginous portion, the mesial end of its oral limb projecting mesially beyond the cartilaginous portion of the fenestra and there lying largely external to the fenestra and hence outside the nasal capsule. That part of the ala nasalis which lies against the inner edge of the cartilaginous portion of the fenestra is there strongly attached to the inner surface of the nasal capsule by connective tissues, but it is nowhere fused with the capsule. It lies against the internal surface of the inner lining membrane of the capsule and is strongly attached to it, and this membrane, in my preserved specimens, lies closely against the membrane forming the membranous postero-mesial portion of the capsule. The two membranes can, however, be easily separated from each other, and it is to the inner lining membrane and not to the outer that the ala nasalis is here attached. It is furthermore this inner lining membrane of the capsule which alone connects the free mesial ends of the oral and aboral limbs of the ala nasalis, no membrane representing an unchondrified portion of the nasal capsule, such as Gegenbaur ('72) describes in this and others of the Plagiostomi, existing here. There is however a stout thick membrane, which doubtless includes the perichondrial membrane, which lies closely upon the external surfaces of the nasal capsule and the ala nasalis, thus binding them together, but this membrane is not an unchondrified portion of either of these cartilages and can be easily stripped from them.

The lateral portion of the ala nasalis, together with the processes α , α' and β of Gegenbaur's descriptions, encircles the in-

current nasal aperture, the process α not however entering the nasal flap or being capable of being turned back as shown in Gegenbaur's figure 6, plate 17. The process α' of this figure of Gegenbaur's projects internally and orally into the nasal capsule, and, together with the adjoining lateral portion of the ala nasalis, forms a broad plate which supports the internal and aboral surface of the incurent aperture, the aperture thus being a short funnel-shaped passage which inclines from without orally and is considerably contracted internally. The excurrent aperture is a similar short and funnel-shaped passage, but the funnel is here inverted, the smaller end lying at the external end of the passage, and the passage is directed from without aborally and hence in the opposite direction to the incurent passage. The external and aboral wall of the excurrent passage is supported by a broad and stout plate which forms that part of the aboral limb of the ala nasalis that lies mesial to the process α , the oral edge of the passage being bounded by that short portion of the oral limb of the ala nasalis which lies mesial to the process β . A short prong-like process rises from the outer edge of the ala nasalis immediately lateral to the process α , and, projecting ventrally (externally) and aborally, abuts against the internal surface of the nasal latero-sensory canal, apparently being developed in supporting relation to that canal.

The nasal flap arises from the free orally directed edge of the process α of Gegenbaur's figure, and from the corresponding edge of that part of the ala nasalis which lies mesial to that process, and, projecting laterally and orally, rests upon the tissues covering the base of the process β . The processes α' and β project inward toward each other and are each clothed with mucous membrane which is prolonged mesially beyond the process, the mucous folds thus formed being apposed, valve-like, so as to form, in part, a roof to the Schneiderian membrane and, in part, to separate the two nasal passages from each other. The base of the process α is connected with the process α' by a thin fold of mucous tissue, this still farther separating the two nasal passages from each other.

The current of water which enters the incurrent nasal aperture is accordingly at first directed against the oral wall of the nasal capsule, then turned aborally and mesially internal to the nasal valve, in the direction of the long axis of the fenestra nasalis and hence in the direction also of the median raphe of the Schneiderian membrane, and then again turned orally in order to issue through the excurrent aperture. The current thus has a zig-zag course through the nasal capsule, entering it at the oral and lateral end of the fenestra nasalis and leaving it at its mesial and aboral end.

The secondary upper lips of this fish lie, as in *Chlamydoselachus*, oral to the nasal apertures, and, as also in that fish, they do not extend to the median line.

In *Heptanchus* the two nasal apertures lie near the lateral edge of the ventral surface of the snout, the incurrent aperture approximately lateral to the excurrent one. In *Chlamydoselachus* the two apertures lie still farther laterally, the incurrent aperture lying dorsal to the lateral edge of the snout and the excurrent aperture ventral to that edge. The positions of these apertures in these two fishes might accordingly be considered to represent two stages in a migration of the apertures from the ventral to the dorsal surface of the snout, such as is found in the ontogenetic development of the Teleostei (His, '92 b), but this is quite certainly not the case, for this change in position of the apertures in the Teleostei is apparently due wholly to an unrolling of the cranial flexure, and not to a migration of the apertures, while the change in position in the Selachii, such as it is, is due wholly to their actual migration.

In both *Heptanchus* and *Chlamydoselachus*, the median raphe of the Schneiderian membrane crosses the line of the external nasal apertures almost at a right angle, running forward and slightly mesially, approximately parallel to the upper edge of the mouth. In both fishes also the processes α and α' of the ala nasalis are fused, the process α forming the external edge of the combined processes; and this combined process and the process β project into the nasal capsule and form, together with that part of the ala nasalis which bounds the lateral (dorsal in *Chlamydoselachus*) half of the incurrent aperture, a broad cylin-

dricul band, slit along the surface presented toward the excurrent aperture. The two edges of this slit are apposed and form the nasal valve and its valve-seat. Short horn-shaped processes arise from the mesial (ventral in *Chlamydoselachus*) surface of this cylinder, one on either side of the valvular slit, and, projecting mesially (or ventrally) and curving toward each other, partly surround the excurrent aperture. The mesial (or ventral) ends of these two latter processes are not connected with each other in my specimens of either of these fishes, thus completing the alar ring as shown in Gegenbaur's figure of *Heptanchus*, and the cartilage is nowhere fused with the outer edge of the nasal capsule. The cartilage is strongly attached to the outer edge of the capsule by ordinary connective tissues, and it is also attached to the inner lining membrane of the capsule. In both *Heptanchus* and *Chlamydoselachus* the lateral edge of the nasal flap is attached to the external edge of the process $\alpha\alpha'$, the full length of the process α , and, because of this attachment, there is no passage connecting the two nasal apertures, between the flap and the nasal valve. No part of the *ala nasalis* actually enters the nasal flap, but the process α lies along the internal surface of the lateral edge of the flap.

In *Chlamydoselachus* the secondary upper lips are, as already stated, short, and they lie oral to the nasal apertures. In *Heptanchus* these lips also lie oral to the nasal apertures, but they are much longer than in *Chlamydoselachus*, and, so far as I can tell from my one much dissected specimen, they extend forward to the symphysis and there fuse with each other, a continuous band of the external surface of the head, concentric with the upper edge of the primary cavity of the mouth, thus here being added to that cavity.

In *Scyllium canicula* (fig. 5) I find the *ala nasalis* practically as described by Gegenbaur ('72), the process α lying in the lateral edge of the nasal flap and the process β lying in the lateral edge of a groove which forms the seat for the flap. The nasal valve is formed by a small fold of mucous tissue which projects mesially from the internal surface of the nasal flap and crosses the aboral end of the process β , and there is no cartilaginous process α' de-

veloped in relation to it. The oral limb of the ala nasalis is not prolonged mesially beyond the process β , this apparently being related to the presence of a naso-buccal groove, but the aboral limb of the ala nasalis is thus prolonged beyond the process α and there sends a second long process into the nasal flap, this process and the process α both being thin and flexible. The line joining the centers of the external nasal apertures is more nearly parallel with the median raphe of the Schneiderian membrane than in *Mustelus*, and the current of water passing through the nasal pit does not have the markedly zig-zag course which it has in that fish.

A secondary upper lip is found in normal position in this fish, and extends from the secondary angle of the gape to the lateral edge of the naso-buccal groove, where it ends abruptly against the lateral wall of the groove. The crest of the fold of this lip runs directly toward the process β , which lies in the line of the fold, and a well marked crease, extending from the line of the angle of the gape about half way to the process β , cuts across the definitive lip and separates the crest of the fold of the secondary upper lip from the primary lip. The nasal flap extends to the upper edge of the mouth, completely covering both the postero-mesial nasal aperture and the naso-buccal groove, and its oral edge has the appearance of forming a part of the secondary upper lip. It, however, forms no part of the fold of that lip, as comparison with Müller and Henle's ('41) figures of *Scyllium edwardsii*, *Scyllium catulus*, and *Scyllium africanum* will show, for in these several fishes, notwithstanding that the oral edge of the nasal flap lies not far from the upper edge of the mouth, the fold of the secondary upper lip runs forward, without interruption, oral both to the nasal flap and the nasal apertures, exactly as it does in *Chlamydoselachus* and *Mustelus*. There is no naso-buccal groove in either of these three species of *Scyllium*, the presence of this groove in *Scyllium canicula* thus being related to a nasal flap which extends to the upper edge of the mouth, or, more properly, to the presence of a nasal-flap furrow which has that extent, that furrow lying beneath the nasal flap (Allis, '16).

The naso-buccal groove of *Scyllium canicula* is short, the postero-mesial edge of the nasal capsule lying not far from the upper edge of the mouth. The groove is bounded laterally by the abruptly ending anterior end of the fold of the secondary upper lip, and bounded mesially by the base of the nasal flap. The postero-mesial nasal aperture has the full width of the naso-buccal groove, and, as the passage leading from the nasal pit to this aperture is always directed orally, the oral edge of the aperture must have lain primarily near the upper edge of the mouth. It is therefore this edge of this aperture that primarily interrupted the fold of the secondary upper lip as it pushed forward toward the symphysis, and the encounter of the fold with the aperture raised the lateral edge of the aperture to such an extent that the oral edge of the aperture became a groove, the mesial edge of the groove being formed by the mesial edge of the nasal-flap furrow. The fold of the secondary upper lip could not cross the postero-mesial aperture and reappear mesial to it, because of the barrier formed by the nasal flap. The naso-buccal groove of this fish is thus not an independently developed structure especially designed to connect the nasal pit and the cavity of the mouth, as is generally assumed to be the case, but is simply the oral edge of the postero-mesial nasal aperture and the corresponding edge of the nasal-flap furrow transformed into a groove by the encounter of the fold of the secondary upper lip with the lateral edge of the nasal aperture.

In *Raia* (species not given) Gegenbaur ('72) shows the ala nasalis completely fused with the outer edge of the nasal capsule. In two specimens of *Raia clavata* I find it wholly separate from the capsule, but strongly attached to the inner lining membrane of the capsule. The process α is long and flexible and lies in the lateral edge of the nasal flap, as shown in the figures in my work on the labial cartilages of this fish (Allis, '16). On the internal surface of this part of the nasal flap is a large pad of tissue, the thicker, aboral portion of which lies directly above the nasal pit while the less tall, oral portion rests in a depression on the opposite side of the nasal opening, immediately mesial to the base of the process β . The process β projects into the

nasal capsule and supports a fold of mucous tissue which represents one-half of the nasal valve. On the opposite side of the nasal opening, and beneath the nasal flap, is another mucous fold, which forms the other half of the nasal valve, but is not supported by cartilage. These two halves of the nasal valve do not, in my specimen, meet in the median line of the nasal pit, but they and the thicker part of the pad on the internal surface of the lateral edge of the nasal flap together form a partition across the pit. In contact with the lateral edge of the ala nasalis, but not fused with it, a narrow band of cartilage arises, and running inward, is at first closely attached to the lining membrane of the capsule, but soon separates from that membrane and lies in relation to the median raphe of the Schneiderian membrane. This raphe is directed mesially and slightly orally, and coincides, in direction, with the line of the external nasal apertures. The postero-mesial edge of the nasal capsule is membranous, and the naso-buccal groove passes over this membranous portion of the capsule.

The naso-buccal groove is large, and extends orally and slightly laterally from the postero-mesial nasal aperture to the anterior edge of the mouth. In my work on the labial cartilages of this fish (Allis, '16) I called this groove the nasal-flap furrow, the naso-buccal groove being considered to be a secondary differentiation of this furrow and to be represented in a deeper, lateral portion of the entire furrow. My present work confirms the opinion there expressed that the entire groove is primarily derived from the nasal-flap furrow, and that the lateral and deeper portion of the groove is a secondary differentiation, but comparison with the conditions in *Scyllium canicula*, as now interpreted, shows that it is the mesial portion only of the entire groove which is derived from the nasal-flap furrow, the deeper, lateral portion of the groove being formed by the crease which, in *Scyllium*, separates the crest of the fold of the secondary upper lip from the primary lip, together with the maxillary preangular labial crease. If these two creases of *Scyllium* were to coalesce and then be extended forward until they fell into the naso-buccal groove of that fish, the naso-buccal groove of *Raia* would be

formed. The conditions in the two fishes are so strictly similar that it seems to me there can be no possible doubt of this, and the formation of the groove in *Raia* is related, as it is in *Scyllium*, to a nasal-flap furrow which extends to the upper edge of the mouth. The fold of the secondary upper lip does not however, in *Raia*, abut against the naso-buccal groove and end there, as it does in *Scyllium*, for it has been deflected from its forward course by the coalescence, with the naso-buccal groove, of the crease between the secondary and primary upper lips. The crest of the fold of this lip accordingly retains its normal relations to this crease and runs aborally along the lateral edge of the groove. In those of the *Raiidae* in which the nasal-flap furrow does not extend to the upper edge of the mouth there is no naso-buccal groove, and the secondary upper lip runs forward, oral to both nasal apertures, exactly as it does in those *Selachii* in which this groove is not found.

In *Heterodontus* (probably *francisci*), a single specimen of which I have had at my disposal (figs. 6 and 7), the outer end of the line of the angle of the gape lies at a relatively considerable distance from the lateral edge of the palatoquadrate, and it has been carried forward into the transverse plane of the hind end of the nasal capsule, or even slightly anterior to that plane. Because of this shortening of the length of the gape without a corresponding shortening of the line of the angle of the gape, the fold of the secondary upper lip is tall, and in running forward, it immediately reaches the process β and is there directed between the two nasal apertures. On the mesial side of the nasal apertures the line of this secondary fold is continued by a well developed fronto-nasal flap, or so-called process. A well developed primary upper lip runs forward along the external edge of the palatoquadrate dental arcade until it has passed the postero-mesial nasal aperture, where the fold spreads out on the internal surface of the fronto-nasal flap and vanishes as a distinct fold. The edge of the fronto-nasal flap is certainly not formed, in any part, by this lip, and it must accordingly either represent an anterior continuation of the crest of the fold of the secondary upper lip, or be a special and independent formation.

Its position, definitely in the line of the fold of the secondary upper lip, is strongly in favor of its being an anterior continuation of that fold, and such I consider it to be, since it is not found in any fish I know of in which the fold of the secondary upper lip has not been interrupted by meeting some part of the nasal groove. The fold of the secondary lower lip is continued forward approximately to the level of the anterior end of the distinctly evident portion of the primary upper lip, and from there onward the lower lip of the fish is a primary one.

The fenestra nasalis of this fish is long and narrow, and its long axis is directed from in front postero-laterally, approximately parallel to the secondary upper lip and hence diverging laterally both from the primary upper lip and the lateral edge of the palatoquadrate. The incurrent nasal aperture lies aboral to the line of the secondary upper lip, the excurrent aperture oral to that line; and the planes of the two apertures are inclined to each other to such an extent that the internal angle between the two planes is less than a right angle. The two apertures are incompletely separated from each other, as in the other Selachii, by an incompletely formed nasal bridge, which lies in the line of the crest of the fold of the secondary upper lip. The excurrent aperture is surrounded by a frill of dermal tissues, apparently a modification of the nasal flap, the frill being continued, as a fold, partly around the incurrent aperture. The nasal section of the buccalis latero-sensory canal passes oral to this frill, between it and the lateral edge of the palatoquadrate, thus encircling the oral edge of the excurrent aperture, as it does in all other Plagiostomi.

The excurrent nasal aperture is thus enclosed within the buccal cavity when the mouth is closed, but it lies definitely between the secondary and primary upper lips, aboral to the latter, and hence in the same relation to it, to the palatoquadrate dental arcade, to the buccalis latero-sensory canal, and to the incurrent aperture which the corresponding aperture has in the other Plagiostomi considered above. There is accordingly no possible doubt that the excurrent aperture of *Heterodontus* is the strict homologue of the corresponding aperture in other

Plagiostomi, that it lies on what is, in them, a part of the external surface of the snout, which has here been secondarily included in the buccal cavity. There is no naso-buccal groove connecting this aperture with the upper edge of the primary cavity of the mouth. The fold of the secondary upper lip, passing as it does between the two nasal apertures, might be considered to correspond to the lateral edge of the snout of *Chlamydoselachus*, which also passes between the two apertures, but these two edges are not homologous, for they both exist contemporaneously and independently in *Chlamydoselachus*.

In *Ginglymostoma concolor* and *Stegostoma fasciatum* the fold of the secondary upper lip has, as shown in Müller and Henle's ('41) figures, approximately the course which it has in *Heterodontus*, but it apparently crosses the nasal pit slightly oral to the process β , and the fronto-nasal flap is not so well defined as in *Heterodontus*.

It is commonly said of *Heterodontus*, that the nasal and buccal cavities are confluent, that the two nasal apertures are connected by a naso-buccal groove, or that the excurrent aperture has shifted orally until it has cut through the upper lip and so come to lie on the internal surface of the lip; the upper lip and the buccal cavity of this fish being considered to be the strict homologues of the lip and cavity of other Plagiostomi. These assumptions are, however, all incorrect.

Huxley ('76) considered the excurrent aperture of this fish as formed by the incomplete bridging of a naso-buccal groove, and he compared it with the posterior nasal aperture of *Ceratodus*. This implies two assumptions; first, that this aperture of *Heterodontus* represents the oral end of a naso-buccal groove which has been incompletely bridged by the arching over of its opposite edges; and, second, that it is the homologue of the posterior aperture of *Ceratodus*, the latter aperture then being the oral end of a canal formed by the completed bridging of a naso-buccal groove similar to the one assumed to be found in *Heterodontus*. The first of these two assumptions is incorrect, as explained above. The second assumption is probably correct in so far as the homology of the posterior nasal apertures of *Heterodontus* and

Ceratodus are concerned, but incorrect as to the formation of this aperture, in the latter fish, by the bridging of a naso-buccal groove, as Greil ('13) has shown and as will be fully discussed later. Furthermore, it may here be stated that the assumption, frequently made, that the bridging of a naso-buccal groove, as that groove is currently described in certain of the adult Plagiostomi and in embryos of these and other vertebrates, could produce two nasal apertures the homologues of those actually found in the adult gnathostome fish is an error. The naso-buccal groove, as described both in the adult and in embryos, is said to extend either from the oral edge of the oral (posterior) nasal aperture, or from that edge of the nasal pit, to the upper edge of the mouth, the primitive oral (posterior) nasal aperture accordingly lying aboral to the aboral end of the groove, and between that end of the groove and the incompletely formed nasal bridge. If then this nasal bridge were to be completely formed, and the naso-buccal groove were to be bridged by the fusion of its opposite edges, the fusion of this bridge with the nasal bridge would give rise to a secondary posterior nasal aperture which would not be the homologue of the aperture actually found in fishes, while the formation of a naso-buccal bridge alone, without the formation of a proper nasal bridge, would give rise to an external nasal aperture which would correspond to the undivided primitive single opening of the nasal pit, and to an internal aperture which would have no homologue in fishes.

The *ala nasalis* of *Heterodontus* (*Cestracion*) *philippi* has been carefully described and figured by Gegenbaur ('72), and it is said by him to be a complete ring, surrounding both nasal apertures, and quite extensively fused, at two points, with the cartilage of the nasal capsule. Huxley ('76) also described and figured this cartilage in this fish, but as a partial and not a complete ring, and he makes no mention of its being anywhere fused with the edge of the nasal capsule; both of which details are in accord with his conclusion that this cartilage is an upper labial cartilage. Daniel ('15) has also described and figured this cartilage in *Heterodontus francisci*, and he also does not find it

either a complete ring or anywhere fused with the edge of the nasal capsule.

In my specimen of *Heterodontus francisci* the fenestra nasalis is, as already stated, a long and relatively narrow opening, and the nasal capsule is relatively deep. The oral and mesial walls of the capsule are largely membranous, much as shown in Gegenbaur's figure 2, plate 16, but unfortunately this membrane had been dissected away along the edge of the ala nasalis before my attention was called to the importance of preserving it, and I can not tell whether it extended to that cartilage, as Gegenbaur states, or not. The conditions in the other Plagiostomi examined would however indicate that it did not. The median raphe of the Schneiderian membrane lies in the line of the long axis of the fenestra nasalis, and the folds of that membrane are so long that they extend outward almost to the inner edge of the ala nasalis. The membrane is, as in the other Plagiostomi, attached to the ala nasalis, and it is so closely applied to the inner surface of the cartilaginous portion of the fenestra nasalis that where it projects beyond the fenestra it appears as an outward membranous extension of the walls of the nasal capsule, and this may be what led Gegenbaur to conclude that the membranous portions of the capsule are directly attached to the ala nasalis.

The ala nasalis is, in my specimen, a complete ring, the postero-mesial portion of which is thin and flexible, and it is completely fused at one point with the outer edge of the nasal capsule. The processes α and β are as described and figured by Gegenbaur and Daniel, and there are projecting mucous folds which form the nasal valve and its seat, but they are without cartilaginous support. The incurrent passage is directed orally, passes through that part of the alar ring which lies lateral to the processes α and β , and leads to the postero-lateral end of the fenestra nasalis. The excurrent passage is directed from without aborally, passes through that part of the alar ring which lies mesial to the processes α and β , and leads to the antero-mesial end of the fenestra nasalis. The little process of cartilage, shown in my figure projecting laterally from the

mesial border of the alar ring, supports the external (ventral) wall of this latter passage and corresponds to the flat recurved end of the alar cartilage shown in Daniel's figure of this fish. The point where the alar cartilage is fused with the outer edge of the nasal capsule lies between this little process and the process β .

The ala nasalis of all of the Plagiostomi was considered by Gegenbaur to be a part of the chondrocranium, and not, as J. Müller ('34) had previously concluded, an originally independent skeletal element which had secondarily fused with the outer edge of the nasal capsule. Huxley ('76) and Parker ('76) must both have accepted Müller's view, for they both (Huxley in *Heterodontus* and Parker in *Scyllium* and *Raia*) describe this cartilage as a labial. Gaupp ('06) however considers Gegenbaur's conclusion to be confirmed by conditions in an 8 cm. embryo of *Mustelus*, the Nasenflügelknorpel being said to there be "in kontinuierlicher Verbindung mit der Nasenknorpel." In a 122 mm. embryo of *Mustelus vulgaris* I find the cartilage everywhere definitely and distinctly separate from the nasal capsule, but the two cartilages are connected by a line of tissue which is a continuation of two thin layers of tissue which are closely applied, one to the external and the other to the internal surface of both these cartilages, and which apparently represent thick perichondrial membranes similar to the one that I have described on the external surface of these cartilages in the adult *Mustelus*. In a 55 mm. embryo of this same fish the ala nasalis is also everywhere definitely separate from the capsule, but at one point the two cartilages closely approach each other and are connected by dense tissue continuous with that which lines both surfaces of these cartilages. This tissue does not however here undergo chondrification, for it persists as fibrous or connective tissue in the older embryo. The alar cartilage is thus quite certainly not cut off from the edge of the nasal capsule in the ontogenetic development of this fish, but it is developed in a layer of embryonic tissue which is continuous with that in which the capsule and adjacent portions of the chondrocranium are developed. There seems however no more reason, simply because of this,

to consider the ala nasalis to be cut off from the outer edge of the nasal capsule than there is to consider the vertebrae to be segmented from the hind end of the cranium.

The posterior upper labial of *Heterodontus francisci* projects dorsally and somewhat posteriorly from the angle of the gape, lies against the postero-lateral surface of the nasal capsule, and hence has the position shown in Huxley's figure of *Heterodontus Philippi* and not that in Gegenbaur's figure of the same fish ('72, fig. 3, pl. 12). The anterior upper labial lies antero-mesial to and parallel with the posterior one, as shown in Huxley's figure. Thus both these two labials project dorso-posteriorly from the angle of the gape, instead of anteriorly, as they do in the other Selachii above considered, this apparently being due to the fact that the dorso-posterior ends of these labials were attached posterior to the nasal capsule, and that accordingly, when their ventro-anterior ends were carried forward by the shortening of the gape, the labials swung forward around their dorsal points of attachment as centers and so became directed dorso-posteriorly. Because of this position of the labials, there is no supralabial furrow. A deep postlabial furrow lies internal to the mandibular labial and extends to its anterior end. A small supramandibular furrow also occurs, external to the mandibular labial and appearing as a groove on the external wall of the large postlabial furrow. It is accordingly not seen unless the latter furrow be forced open. There is a maxillary preangular crease in the upper jaw and a corresponding crease in the lower jaw.

HOLOCEPHALI

In the Holocephali the lips and the nasal apertures differ markedly from those in the Plagiostomi. In *Chimaera coliei* (figs. 13 to 16) the long axis of the fenestra nasalis lies in a plane approximately parallel to the lateral edge of the palatoquadrate, as it does in *Heterodontus*, but the line joining the external nasal apertures lies approximately in the same plane, instead of, as in *Heterodontus*, crossing it at a considerable angle. The two nasal apertures are accordingly, one postero-lateral, and the

other antero-mesial, instead of, as in all the Plagiostomi, one antero-lateral and the other postero-mesial, and it is the antero-mesial aperture of *Chimaera* which is currently considered to be, and probably is, the incurrent aperture. The postero-lateral aperture lies not far from the lateral edge of the palatoquadrate, nearer to it than the antero-mesial aperture, and a short nasobuccal groove leads from it to the lateral edge of the palatoquadrate immediately anterior to the inner end of the line of the angle of the gape. The line joining the two nasal apertures lies in a nearly horizontal position, extending from the antero-mesial aperture, laterally and posteriorly and inclining slightly toward the lateral edge of the palatoquadrate. The Schneiderian membrane lies at the bottom of a relatively deep nasal capsule, as an elliptical rosette, the long axis of which lies in a nearly horizontal position, transverse to the axis of the body, and at a marked angle with the line of the nasal apertures, thus having practically the position that it has in most of the Plagiostomi.

The middle portion of the upper lip is formed by a thick pad of tough dermal and subdermal tissues which has the width of the vomerine teeth, this portion forming the premaxillary upper lip of Huxley's ('76) descriptions, and bounding antero-mesially the nasobuccal groove. Along the oral edge of this lip there is usually, but not always, a shallow but well defined sulcus which separates the lip into thin oral and thick aboral portions. The oral portion is certainly a primary upper lip. The aboral portion has the position of a secondary upper lip, but as it is quite certain that it is formed, not by the fold of the secondary lip, but by the oral edge of the nasal flap which has been turned back upon the dorsal surface of the snout and has there coalesced with the external dermis, it will be best to call it the aboral premaxillary lip. The primary lips of opposite sides are directly continuous with each other in the median line. The aboral premaxillary lips are there separated from each other by a long and deep median incisure. At the lateral edge of the premaxillary lip these two parts of the lip turn toward each other and coalesce, thus forming a rounded fold, the oral end of which forms a slightly projecting angle. Beyond this point the deeper portion

of the primary lip continues dorso-posteriorly along the lateral edge of the palatoquadrate, there forming the floor of the naso-buccal groove. This latter groove certainly includes, as in the *Plagiostomi*, a nasal-flap furrow, the mesial edge of which forms a crease beneath the rounded fold arising where the superficial portion of the primary lip turns outward to coalesce with the aboral premaxillary lip. Immediately posterior to this point the lateral edge of the palatoquadrate is overlapped by a little projecting point on the lateral edge of the mandibular dental plate, shown in Dean's figure 102, ('06) of this fish, this point cutting slightly into the primary lip.

The rounded fold formed by the coalescence of the primary and aboral premaxillary upper lips runs at first aborally, and hence in the direction of the mesial edge of the nasal-flap furrow of the *Plagiostomi*, and then turns posteriorly (absymphysially), approximately parallel to the lateral edge of the palatoquadrate, and forms the oral edge of the postero-lateral nasal aperture. At its dorso-posterior end this fold is continuous with the dorsal end of a transverse ridge on the internal surface of the large naso-labial fold, to be described later, which lies, when the naso-labial fold is closed, in a nearly vertical position and hence diverging posteriorly at an angle to the oral edge of the postero-lateral nasal aperture. The aboral edge of the latter aperture begins along the anterior (symphyseal) edge of the transverse ridge on the internal surface of the naso-labial fold, and, running antero-ventrally, forms the oral edge of the large valvular process, to be described below. When the naso-labial fold is closed, the summit of the transverse ridge rests against that part of the primary lip which forms the floor of the naso-buccal groove, and in that position it forms a wall which closes the passage from the postero-lateral nasal aperture into the buccal cavity while still leaving a free passage through the aperture into the nasal pit, and also from the aperture into a canal between the naso-labial fold and the external surface of the valvular process.

Immediately posterior to the point where the transverse ridge on the internal surface of the naso-labial fold reaches the oral edge of that fold, a normal secondary upper lip begins, and from

there to the secondary angle of the gape is formed by the oral edge of the labial portion of the naso-labial fold, which fold is formed by the fusion of a normal labial fold with another, the probable origin of which will be considered later. This large naso-labial fold completely covers the postero-lateral nasal aperture, the naso-buccal groove, and the nasal valvular process, and its antero-mesial edge runs antero-dorso-mesially around the lateral edge of the antero-mesial nasal aperture and then about half way around its dorsal edge. The fold is separated into nasal and labial portions by a slight transverse and vertical furrow on its external surface, which extends orally from the aboral edge of the fold about half way across it. The transverse ridge on the internal surface of the fold lies at the posterior end of its nasal portion, and hence immediately anterior to the vertical furrow referred to above. Anterior to this ridge a smaller transverse ridge abuts against the external surface of the nasal valvular process.

A primary lower lip extends the full length of the primary gape of the mouth, and a secondary lower lip the full length of the secondary gape, each of these lips extending forward to the median line and there being continuous with its fellow of the opposite side.

Aboral to the large naso-labial fold, and aboral also to the antero-mesial nasal aperture, is another dermal fold, continuous in the median line with its fellow of the opposite side, the united ventral edges of the folds of the two sides forming a somewhat semicircular line which arches over and frames the nasal apertures and the mouth. This fold can be called, for reasons to be given later, the supramaxillary fold, the furrow separating it from the underlying tissues being called the supramaxillary furrow. The fold, as here developed, is a characteristic feature of all the Holocephali that I find figured, and in Chimaera it lodges the outer ends of a series of ampullary tubules, which open on the external surface in a line of ampullary pores immediately dorsal (aboral) to the edge of the fold. The latero-sensory canals all lie aboral (dorso-posterior) to the fold and hence aboral also to both the nasal apertures, the antorbital section of the buccalis latero-

sensory canal not passing, as it does in all of the Plagiostomi and Teleostomi, between the upper edge of the mouth and the nasal apertures.

The supramaxillary furrow is always deepest in its posterior portion, diminishing anteriorly to a shallow sulcus, and it varies in depth in different specimens, this apparently being wholly due to a greater or less oral extension of the outer edge of the fold, the fold in some specimens only overlapping the aboral edge of the labial fold while in others it extends beyond the middle line of that fold. The furrow extends dorsally into the tissues of the head, the side walls of the furrow lying parallel to the external surface of the head, and the fold being, in consequence, a thin sheet of integumental tissues.

A postlabial furrow occurs in normal position, internal to the hind end of the labial fold, the end of the fold enclosing the ventral end of a labial cartilage which I consider, with Vetter ('78), to be the mandibular labial, instead of enclosing, as in the *Selachii*, the articulating hind ends of that labial and one or both of the upper labials. Starting from below and running upward, the bottom of the postlabial furrow crosses the internal surface of the mandibular labial, and, on reaching its dorso-posterior edge, closely approaches the bottom of the supramaxillary furrow, but it never, in my specimens, falls directly into that furrow. The outer portions of the two furrows are however here confluent. The postlabial furrow then turns anteriorly and, crossing the external surface of the mandibular labial, reaches a point immediately ventral (symphysial) to the line of articulation of the mandibular and posterior upper labials, the latter labial being the maxillary labial of Vetter's descriptions. There the furrow falls, at nearly a right angle, into the vertical furrow already referred to as separating the large naso-labial fold into nasal and labial portions. This vertical furrow lies in the direction prolonged of the line of articulation of the mandibular and posterior upper labials, and directly external to the line between the supplementary secondary upper and lower lips, to be described later. It extends about half way across the naso-labial fold, and has no homologue in the *Selachii*.

A supramandibular furrow occurs in normal position, external to an anterior process of the mandibular labial, shown in Vetter's figures of this fish. The furrow is deep, its deeper portion ending posteriorly dorsal to the ventro-anterior end of the postlabial furrow, while its larger, superficial portion is confluent with the corresponding portion of the latter furrow. The supramandibular fold differs from that in the Selachii in that its hind end has been pushed downward on to the external surface of the mandible, this being associated with a change in direction of the line of the angle of the gape, which, in the Plagiostomi, is directed from within antero-laterally. In Chimaera it is directed ventrally and but slightly laterally, the inner end of the line having been carried upward and forward, while its outer end has dropped downward upon the external surface of the mandible. The mandibular preangular labial crease starts from near the inner end of this line and, after running at first symphysially along the lateral edge of the primary lower lip, turns outward across the outer edge of the secondary lower lip to a point dorsal (oral) to the anterior end of the supramandibular furrow. There the anterior end of the crease and furrow are connected by a slight depression in the dermis. This crease thus cuts out of the secondary lower lip an important preangular portion which corresponds to the little islet cut out of this lip by the crease in *Mustelus*. The labial fold overlaps the larger part of this islet, the part so overlapped being thinner than the part beyond it and being separated from it by a low ledge. This ledge corresponds to the outer edge of the secondary lower lip of the Selachii, and forms the edge of the functional lip of Chimaera when the mouth is widely opened; but it is not the functional lower lip when the mouth is closed, the functional lip then being formed by the mesial edge of the preangular islet. In other words, the posterior portion of the secondary lower lip has been turned downward upon the outer surface of the mandible, and a broad and V-shaped portion of its inner surface is presented externally. The angle of the V is directed symphysially, and its mesial arm forms the functional lip when the mouth is closed and the lateral arm the functional lip when the mouth is opened. The former

lip may be called the supplementary, and the latter the actual secondary lower lip.

When the mouth is closed, the supplementary lower lip, as above defined, abuts against the posterior surface of the larger of the two transverse ridges on the internal surface of the naso-labial fold, this posterior surface, abutting as it does against the supplementary secondary lower lip, thus being a supplementary secondary upper lip. The corresponding portion of the actual secondary upper lip is formed, as already stated, by the ventral edge of the labial portion of the naso-labial fold, and it extends forward from the secondary angle of the gape to the vertical furrow which separates this portion of the fold from the nasal portion. Anterior to this vertical furrow the line of the secondary upper lip is continued onward along the rounded ventral edge of the nasal portion of the naso-labial fold, but this edge, although, like the oral edge of the nasal flap in *Scyllium* and *Raia*, it forms part of the upper edge of the mouth, is no morphological part of the fold of the secondary upper lip. The vertical furrow on the external surface of the naso-labial fold lies directly external to the line between the short supplementary secondary upper and lower lips, and has evidently been retained, though, as will be later explained, probably not caused, by the tissues of the thin labial fold there being creased by falling slightly in between the two lips.

The supplementary secondary upper and lower lips form the bounding side wall of the buccal cavity when the mouth is closed, a supplementary gape of the mouth, which lies between the primary and secondary gapes, thus being formed. The line of this supplementary gape runs dorso-posteriorly at a considerable angle to the line of the gape of the jaws, its inner end turning dorsally and but slightly posteriorly, and the mandibular and posterior upper labials articulate with each other immediately dorsal (morphologically posterior) to the inner end of the line. These labials thus lie not far from the inner end of the line of the angle of the gape, instead of near the outer end, as in the *Plagiostomi*, and they lie, when the mouth is closed, external to the palatoquadrate. The mandibular labial has, in consequence,

been pulled upward through the hind end of the labial fold, and its ventro-anterior end, instead of its dorso-posterior end, lies in the hind end of that fold.

The nasal apertures of *Chimaera* are separated from each other by a stout broad valvular process, already several times referred to, which projects ventro-antero-mesially from the aboral margin of the nasal pit. The external surface of this valvular process is concave, and its outer end is also concave or V-shaped, this end fitting against a corresponding surface on the dorso-lateral corner of the thick premaxillary lip of the fish, which forms the valve-seat process. On the internal surface of the valvular process there is a longitudinal ridge which fits into a corresponding depression on the premaxillary lip, a second valvular surface thus being formed which lies ventro-lateral and internal to the V-shaped valvular surface. Because of the markedly concave external surface of the valvular process, a relatively large passage is left between it and the overlapping naso-labial fold, which communicates at one end with the postero-lateral nasal aperture, and at the other end with both the antero-mesial aperture and the exterior. This passage is so large that it would seem as if it must give regular passage to water, either incurrent or excurrent but experiments on the living fish can alone decide this. The posterior edge of the valvular process is formed by a delicate fold of mucous tissue which encloses a delicate piece of cartilage, the cartilage '1' of Hubrecht's ('77) descriptions. There is, in all my specimens, a small teat-like eminence on the mesial wall of the antero-mesial nasal aperture, the possible significance of which will be explained later.

The antero-mesial nasal aperture is encircled by the cartilage 'kn' of Hubrecht's descriptions of *Chimaera monstrosa*, called by him the 'Nasenmuschel' and certainly corresponding to some part of the ala nasalis of the Plagiostomi. In *Chimaera coliei*, this alar cartilage has the form of an oblique section of a cylinder, the axis of the cylinder lying approximately in the line of the trabeculae and hence at a marked angle to the plane of the fenestra nasalis, this giving to the cartilage the appearance of having been pulled forward and upward, almost completely out

of the capsule. It is slightly concave on its dorso-lateral surface, the concavity being bounded dorso-posteriorly by a slight eminence on the cartilage and ventro-anteriorly by two sharply pointed processes of the cartilage. The rounded eminence is bound by ligamentous tissues to a corresponding eminence on the internal surface of the cartilage 'fg' of Hubrecht's descriptions, and the two pointed processes support the tissues of the V-shaped valvular surface of the valvular process. Ventral to those pointed processes the cylinder is slit its full length, the cut edges of the cartilage supporting the longitudinal valvular surface and its valve-seat. The alar cartilage projects slightly into the nasal capsule, the inner lining membrane of which is firmly attached to it.

The conditions in *Chimaera* are thus, as already stated, markedly different from those in the *Plagiostomi*, but it would nevertheless seem as if they could have been derived from those in certain of the latter fishes. In Müller and Henle's ('41) figure of *Chiloscyllium punctatum*, reproduced in the accompanying figure 4, a dermal fold is shown which encircles the lateral edge of the antero-lateral nasal aperture; it may be referred to as the nasal fold in order to distinguish it from the nasal flap. The antero-mesial end of this fold lies anterior (aboral) to the nasal flap and the related process α of the ala nasalis. Posterior to this fold, and lying slightly deeper than it, is the flap-seat and the related process β of the ala nasalis, and posterior to the flap-seat there is a labial fold, the supralabial furrow and the furrow of the nasal fold apparently being continuous at their adjoining ends. In this fish the long axis of the fenestra nasalis has certainly rotated a certain distance in the same direction that it rotates in other *Plagiostomi*, and if this rotation were to be continued until the axis of the fenestra had acquired the position that it has in *Chimaera*, and approximately has in *Heterodontus*, and if each of the two nasal apertures were to follow that end of the axis of the fenestra to which it is related until it came to lie directly external to it, as the two apertures approximately do in *Chimaera*, the antero-lateral aperture would pass internal to the nasal fold, and the nasal

flap and its related process α would acquire the position of the large valvular process of *Chimaera*. The position of the flap-seat and the process β would not be affected by this change in position of the nasal flap, but the antero-lateral nasal aperture would be distorted and would lie somewhat perpendicular to the external surface, a passage leading aborally and mesially from it to the exterior beneath the overlapping nasal fold. An increased development of the nasal fold would then produce the nasolabial fold of *Chimaera*, and the flap-seat (process β) of *Chiloscyllium* would become the transverse ridge on the internal surface of the fold of *Chimaera*. The postero-mesial nasal aperture would, in the meantime, have been crowded mesially beneath the nasal flap and would push it backward, much as it is shown artificially pushed back on one side of Müller and Henle's figure of *Chiloscyllium*, and the process α , as shown in that figure, would swing orally and then mesially until it came in contact with the base of the turned back nasal flap, and a new flap-seat would be formed there.

If the nasal flap, turned back as above assumed, were to fuse with that part of the external surface of the snout which lies beneath it, the edge of the flap would in part form what I have described as the aboral premaxillary lip, and the median incisure of that lip would represent the line of incomplete fusion of the flaps of opposite sides of the head. The remainder of the edge of the flap would encircle a part of the original postero-mesial nasal aperture, separating from it a new and smaller aperture, the anterior-mesial aperture of the adult *Chimaera*. The postero-lateral nasal aperture of the adult *Chimaera* would then be formed by the naso-buccal groove of *Chiloscyllium*; that is, by the oral edge of the original postero-mesial aperture together with the nasal-flap furrow. This postero-lateral aperture would lie internal to the persisting antero-lateral aperture, these two apertures having one edge in common, formed by the postero-lateral edge of the large valvular process (process α), while the other edges of the two apertures would be formed, in the one case by the little fold which crosses the floor of the naso-buccal groove of *Chimaera* and in the other by the transverse ridge on the

internal surface of the naso-labial fold of that fish. The fold of the secondary upper lip of *Chimaera* would then have been interrupted, as it is in the Plagiostomi, by its encounter with the original postero-mesial nasal aperture and not by its encounter with the antero-lateral aperture, as it would have been if that aperture of the Plagiostomi were, as Hubrecht considered it to be, the homologue of the postero-lateral aperture of *Chimaera*. The folding back of the nasal flap would give rise, as already stated, to the rounded fold at the lateral end of the thick premaxillary upper lip of *Chimaera*, and the latero-oral corner of the fold might have been perpetuated in the little teat-like eminence on the mesial surface of the newly formed antero-mesial nasal aperture.

The ala nasalis would naturally undergo modifications during these changes in the nasal apertures, that part of it which encircled the original antero-lateral aperture undergoing reduction, and the part which encircles the newly formed antero-mesial aperture undergoing special development. This latter aperture would be external and aboral to the position occupied by the original postero-mesial aperture, and that part of the ala nasalis which encircles it (cartilage 'kn') would have the appearance of having been pulled or stretched upward and outward from the nasal capsule, as it actually has in *Chimaera*. A remnant of the process β would remain in the ridge on the internal surface of the naso-labial fold, and such a remnant is actually there found. The process α' would become the longitudinal valvular surface, found, in *Chimaera*, on the internal surface of the large valvular process (process α), and it would acquire a new valve-seat near the lateral edge of the newly formed premaxillary lip. The cartilage 'l' of Hubrecht's descriptions, above referred to, and possibly also the little eminence on the dorsal surface of the ala nasalis (cartilage 'kn'), would represent persisting remnants of that part of the ala nasalis which originally encircled the antero-lateral nasal aperture.

During these changes in position of the nasal apertures, the antero-lateral and incurrent aperture would remain connected with the exterior through the passage between the external sur-

face of the process α and the overlapping naso-labial fold, and would remain an incurrent aperture until such time as the originally excurrent aperture had acquired its definitive position and so become better situated to receive the inflowing current of water; provided, of course, that this aperture of Chimaera is actually incurrent and not still excurrent. The furrow related to the nasal fold would probably become the supramaxillary furrow, and if the bottom of this furrow were directed aborally, it would give rise to a supramaxillary fold which would partly overlap the labial fold, as it actually does in Chimaera. The space in chiloscyllium, between the nasal and labial folds would mark the place of, or actually represent, the vertical furrow on the external surface of the nasal-labial fold of Chimaera.

How these changes could affect the buccalis latero-sensory canal to such an extent as to deflect it from its normal course and turn it aboral to the nasal apertures is not apparent, but the cause, whatever it may have been, must have also been operative in the Dipneusti, for this sensory line there also passes aboral to both the nasal apertures. In the Amphibia this sensory line always lies aboral to the posterior (internal) nasal aperture and apparently usually aboral to the anterior (external) aperture also, but the descriptions that I find are not definite as to this.

TELEOSTOMI

In the Teleostomi the nasal pit is said (His, '92 b) to lie, in embryos, on the ventral surface of the snout, as it does in the Plagiostomi and Holocephali. Peter ('06) says that the pit develops late in these fishes, and that when the two nasal apertures later shift from the ventral to the dorsal surface of the snout they always retain their relative positions in relation to the upper edge of the mouth, the anterior aperture of embryos thus being the posterior aperture of the adult. There is apparently a slight rotation of the line of these apertures in the opposite direction to that in which they rotate in the Plagiostomi, and there may be some rotation of the line of the median raphe of the Schneiderian membrane, for Burne ('09)

says that this raphe is sometimes transverse to the line of the apertures. The aboral, or posterior nasal aperture of the adult Teleostei thus corresponds to the aboral, or antero-lateral aperture of the adult Plagiostomi, and the current of water through the apertures of the former fishes is the reverse of that in the latter. The nasal pit is said by Burne to be completely bridged in nearly all, but not all, of the Teleostei, and this bridge is currently considered to be the homologue of the two half bridges of the Plagiostomi fused with each other above the nasal groove. No alar cartilage is however ever found, so far as I know, related to this bridge in these fishes. Burne describes mucous folds which project inward from the internal surface of the nasal bridge and that would seem to correspond to the nasal valves of the Plagiostomi.

In the Holostei and Crossopterygii the nasal apertures and nasal bridge are apparently strictly similar to those in the Teleostei.

Labial and supramandibular folds and furrows are well developed in many if not in all of the Teleostei, Holostei and Crossopterygii, and I have, in an earlier work (Allis, '00), described them in certain of these fishes. The secondary upper lip is represented, in all of these fishes, in the ventral edge of the labial fold, and it is always continuous in the median line with its fellow of the opposite side. It passes between the nasal apertures and the upper edge of the primary cavity of the mouth, and the space included between it and the primary lip forms a secondary addition to the buccal cavity. The maxillary and premaxillary bones lie in this secondary upper lip, as do the labial cartilages of the Selachii in the secondary upper lips of those fishes, and, where teeth are developed in relation to these bones, they form a dental arcade which lies external to and concentric with the primary, palatoquadrate arcade.

A supramaxillary fold is found, more or less developed in many of these same fishes, the related furrow there extending upward internal to the lacrimal bone, or to it and the anterior suborbital bone. This furrow I have already described in Scomber (Allis, '03, p. 64) as an important fold which "extends

upward between the outer surfaces of the maxillary and premaxillary and the inner surface of the lachrymal," but I did not then recognize that the projecting fold which encloses the ventral edge of the lacrimal bone had any morphological significance. It is, however, quite unquestionably the homologue of the supramaxillary fold of the Holocephali, the fold in the one being related to a latero-sensory line and in the others to an ampullary line, for the line of ampullary pores related to this fold in *Chimaera* marks the primitive position of the related ampullary sacs (Allis '01). In *Amia* this fold and furrow are much less developed than in *Scomber*, but they are both still related to the lacrimal bone, and the base of the shank of the maxillary bone passes upward in the furrow, internal to the fold. In *Gadus* the fold has been extended forward until it meets in the median line and is there continuous with its fellow of the opposite side, the fold lying internal to the ventral edges of the lacrimal and first suborbital bones. The lacrimal bone here extends far forward between the upper edge of the mouth and the nasal apertures, and is traversed by the buccalis latero-sensory canal. This canal does not extend, in this fish, anterior to the lacrimal bone, the antorbital and dermal ethmoid bones of *Amia*, and the sections of latero-sensory canal related to them, not being found here. The supramaxillary fold is thus related, in both *Gadus* and *Amia*, to the suborbital portion of the buccalis latero-sensory canal and not to its antorbital section, and, as this section of the canal in *Amia* turns upward in the lacrimal bone posterior to the posterior nasal aperture, the supramaxillary fold also turns upward there, while in *Gadus*, because of the different position of the lacrimal bone, the fold runs forward, oral to both nasal apertures. In *Ophidium*, *Merlangus*, *Ammodytes* and *Pleuronectes* I find the supramaxillary fold in the same position as in *Gadus*, and also continuous with its fellow of the opposite side, and as I do not find it so in any of the other Teleostei at my disposal, this is apparently a characteristic of the Anacanthini. The position of the fold would here seem to be determined by the position of the canal, rather than the position of the canal by that of the fold. No teeth are ever found, so far as I know,

developed in relation to the supramaxillary fold, but marked tooth-like spines may be developed in relation to it, as on the lacrimal bone of *Scorpaena* (Allis, '09).

In the Chondrostei the conditions are markedly different from those in the other Teleostomi, and I am unable to give a definite opinion regarding them. In my earlier work (Allis, '00), I came to the conclusion that the labial fold, there called the maxillary fold, was represented in a fold of dermal tissues shown by Parker ('82) extending across the snout of larvae of *Acipenser*, immediately anterior to the barbels of the fish. This fold is not shown either in my figures (Allis, '04) of the adult *Acipenser* or in those of *Scaphirhynchus*, but in both of these fishes the anterior portion of the buccalis latero-sensory canal has approximately the position of the fold in larvae of *Acipenser*. It is therefore probable that this fold in the larvae of *Acipenser* is the supramaxillary fold of the present descriptions, and hence not a maxillary fold as I formerly concluded; and it passes, as the fold does in *Gadus*, between the upper edge of the mouth and the nasal apertures. The primary lips of both *Acipenser* and *Scaphirhynchus* are certainly represented in some important part of the lips of the suctorial mouth of these fishes. Whether or not secondary lips are also represented in some part of the lips I can not determine. If present there, they are quite certainly rudimentary and found only at the angle of the gape, and it may be that they are wholly wanting. In *Polyodon*, also, the secondary lips, if present, are found only at the angle of the gape, this rudimentary condition of the secondary upper lip doubtless accounting for the absence of a premaxillary bone in all these fishes, and for the peculiar position and character of the maxillary splint in *Polyodon*. The conditions in these fishes however need further investigation.

DIPNEUSTI

In embryos of *Ceratodus* the nasal groove, as shown by Semon ('93) and Greil ('13), is, when first formed, directed postero-mesially, as it is in embryos of the *Selachii*, but it later

becomes directed postero-laterally. The anterior end of the groove in the younger embryos shown by these authors apparently corresponds to the same end of the groove in the older ones, the aboral (anterior) nasal aperture of the adult fish (the incurrent aperture), accordingly corresponding to the antero-lateral and also incurrent aperture of the Selachii. The line of the nasal groove shown in the older embryos, which coincides in direction with the line of the nasal apertures of the adult, accordingly rotates in this fish in the opposite direction to that in which it rotates in the Selachii and Holocephali. In the Teleostei the line of the nasal apertures also rotates slightly in this same direction, as already stated, and this is also the case in the Amniota. There must then be some reason for this difference in the direction of rotation of this line in these different vertebrates, and it would seem to be related to the position of the septum nasi, for this septum, when present in the Plagiostomi and Holocephali, lies ventral to the trabeculae, while in *Ceratodus* and in the Teleostomi and Amniota it lies dorsal to the trabeculae.

Günther ('71) says that both the nasal apertures of the adult *Ceratodus* lie within the cavity of the mouth. Huxley ('76) later concluded that "the anterior nares can in no sense be said to open into the cavity of the mouth, inasmuch as they lie outside the premaxillary portion of the upper lip, and are not enclosed by the maxillary portion of that lip. They are not even placed between the upper and the lower lips, inasmuch as the vaulted flap, on the under side of which they lie, is not the upper lip, but the anterior part of the head." Semon ('93) says that, in embryos of this fish, the anterior aperture lies anterior to the upper edge of the mouth and the posterior aperture posterior to that edge, and that the two apertures arise through the coalescence of the opposite lips of a naso-buccal groove. Gegenbaur ('98) says that the posterior aperture, alone, is a choana, the anterior aperture lying on the edge of the lip, and hence not in the cavity of the mouth, and representing the primitive opening of the nasal pit; but he was uncertain as to whether or not the posterior aperture was derived, as he says it is in certain of the Selachii

and in the Holocephali, from the coalescence of the lips of opposite sides of a naso-buccal groove. Greil ('13) says that, in embryos, both apertures lie in the roof of the mouth, that they arise, as in the Teleostei, by the coalescence of the opposite edges of a nasal groove formed by the elongation of the primitive nasal pit, and that no naso-buccal groove is ever developed in this fish.

Huxley ('76) described two cartilages in this fish which he considered to be the homologues of the anterior and posterior upper labials of the Selachii, one of them lying between the anterior and posterior nasal apertures and the other posterior to the latter aperture. Bridge ('98) later concluded that the anterior of these two cartilages was not a labial cartilage, but a persisting remnant of the ventral wall of the nasal capsule, and he called it the subnasal cartilage. The posterior cartilage he was inclined to consider, with Röse ('92), to be the homologue of the so-called antorbital process of *Lepidosiren* and *Protopterus*, but he suggested that it might be the homologue of a wholly separate and independent cartilage, found in the latter fishes, which he considered to be unquestionably the homologue of one of the upper labial cartilages of the Selachii. Fürbringer ('04) accepted Bridge's conclusion regarding the anterior of these two cartilages of *Ceratodus*. Regarding the posterior cartilage, he says that it is the homologue of the upper labial cartilage of Bridge's descriptions of *Lepidosiren* and *Protopterus*, but that this cartilage is, in all these fishes, a detached portion of the chondrocranium and not the homologue of either of the upper labials of the Selachii. Because of this derivation of the cartilage he calls it the postnasal cartilage. He describes and figures a cross-bar of cartilage lying lateral to the posterior nasal aperture and connecting the subnasal and postnasal cartilages, and he says that it is a secondary, protective arrangement, developed in relation to these cartilages and the nasal apertures. Huxley neither describes nor shows this cross-bar of cartilage connecting his two upper labials.

In a large but not well preserved head of this fish I find the cross-bar of cartilage described by Fürbringer, but in my speci-

men it is simply a somewhat posteriorly directed process, or prolongation, of the so-called subnasal cartilage, the outer end of this process reaching and being strongly attached by ligamentous tissues to the so-called postnasal cartilage of Fürbringer's descriptions, but not being continuous with it. The lateral prolongation of the subnasal cartilage beyond this cross-bar, shown in Fürbringer's figure, is, in my specimen, simply a band of tough ligamentous tissue which runs forward along the lateral edge of the anterior nasal aperture and is lost in the tough tissues of the upper lip. The latero-sensory canals of this fish all lie aboral (dorso-posterior) to both the nasal apertures, and aboral also to the upper lip, this being the relations that they have, in the Holocephali, to the nasal apertures and the supramaxillary fold of those fishes.

Comparing the conditions in this fish, as thus described, with those in the Plagiostomi, it seems certain that the anterior and posterior nasal apertures of these fishes are respectively homologous. The so-called subnasal cartilage of *Ceratodus* must then be a remnant of the alar cartilage of the Plagiostomi, for that this cartilage of *Ceratodus*, spanning as it does the primitive single nasal opening, can be a persisting remnant of any part of the walls of the nasal capsule is evidently impossible, those walls encircling the primitive nasal opening and not spanning it. This cartilage is said to be found, in *Lepidosiren*, fused at its lateral end with the edge of the nasal capsule and there appearing as a process of that capsule, and this would be in accord with Gegenbaur's derivation of the alar cartilage from the outer edge of the nasal capsule, but in *Ceratodus* it is an independent cartilage, and this would seem to be its primitive condition. The posterior process of this cartilage, as I find it, the cross-bar of Fürbringer's descriptions, must then also be a part of this alar cartilage, and the ligament which lies along the lateral edge of the anterior nasal aperture, a further but unchondrified portion of it. The postnasal cartilage is, for reasons given immediately below, quite certainly not a part of this alar cartilage, and would seem to be the homologue of the cartilage 'f' of Hubrecht's ('77) descriptions of *Chimaera* and *Callorhynchus*,

this cartilage being said to be fused with a cartilage 'g' in *Chimaera*, but a separate and independent cartilage in *Callorhynchus*. Hubrecht considered both these cartilages of the *Holocephali* to be alar cartilages ('Nasenflügelknorpel'), and Schauinsland ('03) calls them nasal cartilages, but I consider the cartilage 'f' to be an upper labial cartilage.

It is evident that the upper lip of *Ceratodus*, as defined by Günther and confirmed by Greil, corresponds to the supramaxillary fold of *Chimaera*. The upper lip as defined by Huxley was said by him to consist of two parts, one represented in a transverse integumental fold lying immediately anterior to the so-called vomerine teeth, and the other by a fold extending forward from the angle of the gape of the mouth along the lateral edges of the nasal apertures. The former fold, as I find it, I consider to be a part of the primary upper lip of the fish, the other, which is hardly recognizable in my specimen and found only at the angle of the gape, being a secondary lip. The functional upper lip is then, as Huxley concluded, simply a fold of the dermis on the anterior part of the head, and is accordingly a tertiary upper lip lying anterior to the secondary one and circumscribing a second band of the external surface of the head which is here added to and included in the cavity of the mouth.

These lips and the labial folds and furrows of *Ceratodus* are shown in the accompanying figure 12, and it is there seen that there are two angles to the gape of the mouth, one the actual angle and the other the apparent angle when the mouth is closed. The actual angle corresponds to the angle of the secondary lips of the *Selachii*, and immediately posterior to it there is a short flat flap of dermal tissues which is the hind end of the labial fold. This latter fold lies parallel to the dorsal surface of the mouth cavity, and its lateral edge lies slightly internal to the edge of the functional, or tertiary upper lip. Dorsal and posterior to this fold is the external opening of the so-called labial cavity of Günther's descriptions, that cavity lying dorsal to the cavity of the mouth and being the supramaxillary furrow of the present descriptions. In an earlier work (Allis, '00) this furrow was

also called by me the supramaxillary furrow and was said to be the homologue of the similarly named furrow in *Amia*, but I now recognize that there are, in *Amia*, two partly confluent furrows here, one being the labial furrow and the other the supramaxillary furrow of the present descriptions. From the external opening of this furrow the postlabial portion of the labial furrow runs forward in the lower jaw to the hind end of a supramandibular fold, and is there continuous with the supramandibular furrow. When the mouth is closed the functional, or tertiary upper lip extends posteriorly across the actual angle of the gape and ends at the hind end of the supramaxillary furrow, where that furrow and the postlabial furrow are fused in their superficial portions, this point forming a tertiary angle of the gape and lying posterior to the actual, or secondary angle. When the mouth is open, the line of the tertiary upper lip is seen to be interrupted for a short distance immediately posterior to the secondary angle of the gape. The lateral end of the so-called postnasal cartilage of Fürbringer's descriptions passes between the labial cavity (supramaxillary furrow) and the cavity of the mouth, and ends in the mesial edge of the short labial fold. This cartilage is thus quite unquestionably an upper labial which has been pulled postero-mesially out of the labial fold, but there is nothing in this fish definitely to show whether it is an anterior or a posterior upper labial. It, however, evidently corresponds to the cartilage 'f' of *Chimaera*, above referred to, and as that cartilage is certainly not a posterior labial, both it and the cartilage of *ceratodus* must be anterior upper labials.

SUMMARY AND COMPARISONS

It is thus seen that there are three distinctly different types of lips in the gnathostome fishes, a primary lip, a secondary lip, and a tertiary lip, and a band of the external surface of the head is added to the functional cavity of the mouth between the primary and secondary lips and a second such band between the secondary and tertiary lips. The primary lips, as functional

lips, are found only in the antero-mesial portions of the lips of the Plagiostomi, and probably also in those of the Chondrostei. The secondary lips are probably found in the postero-lateral portions of the lips of the Chondrostei; are found either in the corresponding portions only of the lips of the Plagiostomi, or extending the full length of the gape of those fishes; extending the full length of the gape in all of the Teleostei, Holostei and Crossopterygii that I have been able to examine; and extending the full length of the gape in the lower jaw of the Holocephali, but not the full length of the gape in the upper jaw.

The secondary upper lip passes between the upper edge of the mouth and the nasal apertures in all of the Teleostei, Holostei and Crossopterygii that I have been able to examine, as it also does in the Chondrostei, if there present. In all of the Plagiostomi that I have been able to examine in which there is no naso-buccal groove, excepting only *Heterodontus*, the secondary upper lip also passes between the upper edge of the mouth and the nasal apertures; but in those of these fishes in which there is a naso-buccal groove, the lip either abuts against the groove and ends there (*Scyllium*), or runs along the lateral edge of the groove (*Raia*); the naso-buccal groove resulting from the encounter of the fold of the secondary upper lip with the postero-mesial (oral) nasal aperture. In *Heterodontus* the secondary upper lip passes between the two nasal apertures and is continued mesial to those apertures as the so-called fronto-nasal flap, or process. In the Holocephali this lip is represented in the oral edge of the labial portion of the naso-labial fold, and it was primarily interrupted by its encounter with the postero-mesial nasal aperture, as it is in those of the Selachii in which a naso-buccal groove is found.

The tertiary lip is found only in the Dipneusti, and even there only in the upper jaw, the lip passing aboral to both the nasal apertures. In adults of the Holocephali and Teleostomi (excepting the Chondrostei), and probably also in embryos of *Acipenser*, this tertiary upper lip of the Dipneusti is represented in a fold of the dermis on the external surface of the head which is the supramaxillary fold of the present descriptions.

The primary upper lip lies immediately external to the teeth developed in relation to the palatoquadrate. The secondary upper lip lies immediately external to the maxillary and premaxillary teeth. The tertiary upper lip has no teeth developed in relation to it, but in the Teleostei and Holostei the corresponding supramaxillary fold encloses the oral edges of the lacrimal and anterior suborbital bones.

In the Amniota the functional lips are the secondary ones, and the secondary upper lip passes, in all these vertebrates, between the two nasal apertures. Maxillary and premaxillary teeth or bones may be developed, as in the Teleostei, Holostei, and Crossopterygii, in relation to this secondary upper lip. In most of the Sauropsida both of these latter bones are actually developed in relation to this lip, and there are, accordingly, in the upper jaw of these vertebrates, as in the Teleostei, Holostei, and Crossopterygii, two arcades, with or without teeth, an inner and primary arcade formed by the bones developed in relation to the palatoquadrate and an outer and secondary arcade formed by the maxillary and premaxillary bones; and the posterior nasal apertures lie between these two arcades. In certain of the Sauropsida a secondary palate is formed by ventral plates of the vomer and palatine, and the definitive choana lies posterior to the plate so formed, but the primary choana nevertheless still lies anterior to the dorsal and primary portions of those bones. In the Mammalia this same relation of the posterior nasal apertures to the two arcades must also persist, but I am not familiar enough with these vertebrates to discuss the conditions there. Comparison with fishes would however suggest that the presence of a cheek in the Mammalia ditremata is due to a marked reduction of the maxillary bone, as in *Polypterus* (Allis, '00), and its fusion with the pterygoid, this then accounting for the absence in these animals of the latter bone, as claimed by Gaupp ('10). And it may be further mentioned, as a curious coincidence, that a dimple is found in the cheek of man in approximately the position of the postlabial furrow of fishes. In the Mammalia monotremata, where the pterygoid persists in normal reptilian position (Gaupp,

'10), there are said to be no 'lips' (Göppert, '06, p. 79), and hence, of course, no cheek.

In embryos of all of the gnathostome fishes above considered, the primary lips of either side of the head are at first represented in the corresponding half of the edge of the primary stomodaeum. When the so-called maxillary and mandibular processes of either side later begin to develop, they overlies and include the absymphysial portions of this edge of the stomodaeum, leaving the symphysial portions of that edge exposed between their anterior (symphysial) ends and the corresponding ends of the processes of the opposite side, as shown in figures of embryos of all of these fishes. The mandibular processes of opposite sides always, in these figures, ultimately meet and coalesce at the symphysis, but the conditions in the adult show that this can not take place in all of the Plagiostomi. The maxillary processes of opposite sides, on the contrary, do not always meet and coalesce at the symphysis in these embryos, as is well shown in Göppert's ('06) figures of embryos of *Torpedo* and *Mustelus* and Keibel's ('06) figures of embryos of *Acanthias*, a portion of the edge of the primary stomodaeum, which represents a corresponding part of the primary upper lip, always remaining exposed between them and forming the median portion of the definitive upper lip. Keibel ('06, p. 157) calls attention to the fact that, in embryos of *Acanthias*, the middle portion of the upper lip is not formed by the maxillary processes, and, although he could not determine from what it was formed, he questions its origin from the fronto-nasal process. It is, in fact, not formed by that process, properly so-called, for the oral edge of the process is formed by the crest of the fold of the secondary upper lip, and the fronto-nasal process, properly so-called, only occurs where the secondary upper lip crosses some part of the nasal groove and has been cut in two by its encounter with it. This takes place in *Heterodontus*, and probably also in certain others of the Plagiostomi in which there is a naso-buccal groove, but it does not take place in any of the Plagiostomi in which there is no naso-buccal groove, nor in any of the Teleostomi, the secondary upper lip there always passing between the upper edge of the mouth and the

nasal apertures. When, in the adults of any of these fishes, the secondary upper lips meet and coalesce at the symphysis, the maxillary processes also meet there and coalesce, and that band of the external surface of the head which, in earlier embryos, lies between their anterior ends is enclosed in the buccal cavity as a part of the secondary addition to that cavity. If the anterior ends of the maxillary process were to approach each other closely, but not fully to meet and coalesce, a median incisure would be left in the definitive upper lip.

In embryos of all of the Amniota the fold of the secondary upper lip has been cut into maxillary and fronto-nasal portions by its passage across the nasal groove, as it is in certain fishes, and as each of these two portions of the lip, or so-called processes, lies at first oral to the corresponding nasal process, as those processes are defined by Peter ('06), the fold of the secondary upper lip must here have passed across the oral nasal aperture and not between the two apertures. In Mammalian embryos the fronto-nasal and mesial nasal processes later fuse completely with each other to form the *processus globularis* of His's ('92 b) descriptions. This globular process then arches over the nasal groove and fuses with the maxillary and lateral nasal processes, either singly and separately or after those two processes have fused with each other, and a nasal bridge is formed which is certainly the strict homologue of the bridge in the Teleostomi, for the fold of the secondary upper lip can, at the most, simply have caused a widening of the bridge. The fact that, in the Mammalia, the posterior nasal aperture is temporarily closed by the contact of the cut ends of the fold of the secondary upper lip, and that a *membrana bucco-nalis* is formed there and later broken through, is certainly simply a modification of the normal process of development, as found in the Teleostomi, and is due to the fold of the secondary upper lip not passing across the center line of the definitive nasal bridge. Keibel also considers this manner of formation of this aperture of no morphological significance, for he says ('93, p. 477):

so erscheint es mir von Wichtigkeit, dass festgestellt wurde, dass der laterale Stirnfortsatz an der Bildung des primitiven Gaumens beteiligt

ist, ja beim Säuger diese Bildung einleitet. Mindere theoretische Bedeutung kann ich der Thatsache beimessen, dass die primitive Choane erst secundär durchbricht, und dass die Nasenhöhle in ersten Stadium ihrer Entwicklung nicht durch eine Spalte, sondern durch eine solide Epithelleiste mit der Mundhöhle in Verbindung steht. Es handelt sich hier nicht um principielle Verschiedenheiten. Ob die Verbindung zwischen zwei Hohlräumen durch eine Epithelleiste oder durch eine Spalte hergestellt wird, kommt in vielen Fällen im Grunde auf dasselbe hinaus.

The primary lips of embryos of the Amniota are represented, as they are in fishes, in the deeper portions of the mandibular, maxillary and fronto-nasal processes.

In embryos of Amphibia the conditions, as described by authors, are totally different from those above considered. In the Gymnophiona the conditions are simpler than in the Urodela and Anura. In the former (*Hypogeophis*) a large lateral process (laterale Stirnfortsatz) is said by Hinsberg ('02) to project across the oral edge of the nasal pit and to fuse completely, in its deeper portion, with the fronto-nasal process. The superficial portions of the two processes do not however fuse, this leaving, between the processes a shallow groove which extends from the nasal pit to a depression in the roof of the mouth (*Gaumendach*) which lies between the bases of the two processes; and it is important to note that this shallow groove connects with the outer edge of the nasal pit, and not with its deeper portion. The fold of the secondary upper lip passes along the middle line of the lateral process and continues beyond it across the fronto-nasal process, the fold thus lying between the nasal opening and the depression in the roof of the mouth. In slightly older embryos the lateral and fronto-nasal processes project above the shallow groove described above and there again fuse with each other, thus enclosing the groove beneath the epidermis, the nasal end of the groove becoming a closed canal surrounded by epithelial tissues, and its oral end becoming a solid cord of epithelial tissue. The processes do not so project and fuse with each other above the depression in the roof of the mouth, that depression still persisting between the bases of the processes. In still older embryos the lumen in the epithelial cord is prolonged orally,

and finally reaches and opens into the depression in the roof of the mouth, there forming the primitive choana.

The lateral process of these embryos of *Hypogeophis* thus fuses in two places with the fronto-nasal process, one internal to the canal leading from the nasal pit to the primitive choana and the other external to it, the canal thus lying between the two points of fusion and leading primarily from the definitive external nasal opening to the choana without traversing the nasal pit. This canal thus strongly recalls the passage which, in the adult *Chimaera*, lies between the valvular nasal process and the nasal portion of the naso-labial fold and leads from the antero-mesial nasal aperture to the postero-lateral one, and I consider it to be its homologue.

If this be so, the conditions in *Hypogeophis* would be derived directly from those in *Chimaera* by the fusion, first, of the valvular process of *Chimaera* with its valve-seat, this forming a nasal bridge and being represented in *Hypogeophis* by the first of the two fusions of the lateral and fronto-nasal processes, and second, by the fusion of the nasal portion of the naso-labial fold of *Chimaera* with the premaxillary lip, this being represented in *Hypogeophis* by the second of the two fusions of the two processes. By an extension of the first of these two fusions, the postero-lateral nasal aperture of *Chimaera* is occluded, this leaving the naso-buccal groove external to the nasal bridge and an oral remnant of it persisting as the depression in the roof of the mouth of *Hypogeophis*. The large lateral process of embryos of *Hypogeophis* thus represents, in its deeper portion, the lateral nasal process of the *Plagiostomi*, and in its superficial portion the nasal portion of the nasolabial fold of *Chimaera*. The primitive choana of *Hypogeophis* would then correspond to the oral end of the naso-buccal groove of *Chimaera*, and would accordingly be the homologue of the choana of the *Amniota*, but its connection with the nasal pit would be by a canal which passes external to the nasal bridge instead of internal to it.

In the *Urodela* and *Anura* the conditions differ from those in the *Gymnophiona* simply in that the shallow groove which becomes the choanal canal of the latter has been obliterated

instead of being invaginated, this necessitating a secondary reopening of the occluded postero-lateral nasal aperture of Chimaera. The fact that the opening so formed is said (Hinsberg, '01) to lie primarily in the dorsal wall of the anterior end of the alimentary canal, and hence posterior to the oral plate (bucco-pharyngeal membrane), can not affect this homology, for the choana of the adult still lies anterior to the bones developed in relation to the palatoquadrate, and hence quite certainly anterior and not posterior to the primary upper lip, between that lip and the secondary one.

If this be the manner in which the nasal apertures of the Amphibia have been developed, and it seems to me that it must be, the embryological processes simply being obscured by condensations and abbreviations, then the Amphibia must either be descended from some selachian similar to the one from which Chimaera is descended, or directly from some early Chimaeroid. The apparent similarity, in Chimaera and the Amphibia, in the relations of the buccalis latero-sensory line to the nasal apertures, is in favor of the latter assumption, and, furthermore, it seems improbable that these complicated and peculiar nasal apertures would have been twice developed in the vertebrate series. This origin of the Amphibia would also probably explain the palatoquadrate, the upper and lower labials, and the horny jaws of larvae of the Anura.

From the preceding descriptions of embryos and adults, it is evident that the primary lips of all of the gnathostome vertebrates must lie primarily at or but slightly anterior to the oral plate of embryos, for as the mandibular arches lie morphologically posterior to the plate, the cartilaginous bars of those arches must also have primarily had that position. It would then be natural to conclude that the primary upper lips, which lie immediately anterior to the teeth developed in relation to the cartilaginous mandibular bars, are developed from tissues which lie oral to the hypophysial invaginations, but that this is so in all vertebrates can not be definitely determined from the descriptions given of embryos.

Dohrn ('04, fig. 9, pl. 16), in a median sagittal section of an embryo of *Torpedo*, shows what would seem to be the primary upper lip lying posterior to the hypophysis, and it is shown as formed of ectoderm on its external, and of entoderm on its internal surface, as if it were a remnant of the oral plate. His however shows ('92 b, fig. 26), in a median sagittal section of an embryo of *Pristiurus*, a dermal fold immediately anterior (aboral) to the hypophysis which he calls the upper lip of the fish, and in a similar figure (His, '92 a, fig. 14) of an embryo of *Scyllium*, what is apparently this same fold, but not named, is shown in a similar position. There is, however, in both these figures of His's, a larger but lower fold of the ectoderm, oral to the hypophysis, in the place occupied by the fold in Dohrn's figure of *Torpedo*. It may then be that the fold called by His the upper lip in his figure of *Pristiurus* is a secondary and not a primary lip. Comparison with Lundborg's ('94) figures of *Salmo salar* would seem to show that this is the case. In this latter fish Lundborg shows, in median sagittal sections, a large rounded eminence, rather than a fold, immediately anterior (aboral) to the hypophysial invagination, and anterior to it there is a small ectodermal fold projecting postero-ventrally from the posterior surface of the rounded anterior end of the snout. The low and rounded eminence is said to later become a part of the dorsal surface of the buccal cavity, and it seems quite unquestionable that the small ectodermal fold immediately anterior to this eminence becomes the maxillary breathing-valve. The functional upper lip of the fish, which is here unquestionably a secondary one, must then lie anterior to this breathing-valve, and hence must be developed from tissues on the ventral edge of the rounded anterior end of the snout, and it would seem to be shown, in process of development, in His's ('92 b, fig. 31) figure of a sagittal section of a 20 mm. trout. If then the small fold which I take to be the maxillary breathing-valve be that valve and not the primary upper lip, the latter lip must either be represented in the low and rounded eminence which becomes part of the dorsal surface of the buccal cavity, or it must lie posterior to the hypophysis; and as, in the younger embryos figured by

Lundborg, there is a considerable distance between the hypophysial invagination and the oral plate, and as the mandibular branchial bars certainly lie morphologically posterior to the oral plate, it would seem as if the primary upper lip must lie between the plate and the hypophysis.

The conditions in the embryos above referred to thus give conflicting evidence as to the relations, in the Plagiostomi and Teleostei, of the primary and secondary upper lips to the hypophysis, but in embryos of *Amia* and *Acipenser* positive evidence is given of these relations, in these fishes, by Reighard and Mast, and von Kupffer, respectively. In *Amia*, Reighard and Mast ('08) say that the adhesive organ is developed from entoblastic tissues which probably represent the anterior head cavities of the fish, and that this organ only secondarily acquires connection with the ectoblast. This ectoblastic connection, when acquired, lies between the fundament of the hypophysis and the stomodaeum, and the adhesive disk of larvae is developed there; and as my figures of these larvae of *Amia* (Allis, '89) show that the lips of this fish, both primary and secondary, lie oral to the adhesive disk, they must both necessarily develop from tissues oral to the hypophysis. In *Acipenser*, also, the adhesive organ lies between the hypophysis and the stomodaeum (von Kupffer, '93), and as the adhesive organ of embryos is said to become the barbels of the adult (Reighard and Phelps, '08), and as the upper lip of the adult lies oral to these barbels, this lip of this fish, whether it be simply a primary one or a primary and secondary combined, must also lie oral to the hypophysis.

It thus seems certain that the secondary upper lip, at least, varies, in different fishes, in its relations to the fundament of the hypophysis, lying oral to it in the Ganoidei and aboral to it in *Salmo*; and this difference in position is associated with the presence or absence, in embryos of these fishes, of an adhesive organ. The fold of the secondary upper lip must accordingly be developed later than either the hypophysis or the adhesive organ, and, as it pushes forward from the angle of the gape, it passes oral or aboral to the hypophysis accordingly as that organ is more or less remote from the oral plate. Keibel's statement

('06, p. 157) that, in all vertebrates, from the Selachii upward, the upper edge of the mouth lies anterior to the hypophysis, is accordingly not wholly correct.

The primitive invagination of the mouth, or primary stomodaeum, certainly lies posterior to the primary lips, whatever the relations of those lips to the hypophysis may have been, and it is represented only in the deeper portion of the depression enclosed between the so-called mandibular, maxillary and fronto-nasal processes of embryos, the remainder of that depression representing the space between the primary and secondary lips. The stomodaeum of current descriptions of vertebrate embryos is accordingly something more than the primary stomodaeum, being, in all the gnathostome vertebrates excepting the Dipneusti, largely a portion of the external surface of the head which is in process of being secondarily enclosed between the primary and secondary lips, and, in the Dipneusti, being largely a space in process of being enclosed between the primary and tertiary lips.

The Schnauzenfalte of His's ('92 b) descriptions of vertebrate embryos is quite certainly, in certain instances, either the primary or secondary upper lip. In embryos of the Mammalia it has strikingly the position of the median portion of the supra-maxillary fold of the Holocephali and Dipneusti, but it seems probable that it is not that fold but a special formation peculiar to the Mammalia, the supramaxillary fold being limited to the extent that it has in the Teleostomi, and being represented in that portion of the maxillary process of embryos of the Mammalia which bounds the lacrimal groove laterally and which is well shown in Keibel's ('93) figures of embryos of the pig. The fact that the lacrimal canal of the adult mammal lies between the lacrimal bone and the nasal spine of the maxillary bone, and the probability that the lacrimal and antorbital bones of *Amia* represent, respectively, the lacrimal bone and the nasal spine of the Mammalia, is in favor of this supposition, for it is at just this place that the anterior end of the supramaxillary furrow turns upward and ends in *Amia*. If this be so, it seems worthy of note that a furrow beneath a fold definitely related, in the

Holocephali, to the tubules of ampullary sacs which lie on the dorsal surface of the snout, is represented, in the Mammalia, by a groove which later becomes connected with a glandular structure also lying in this region.

The position of the secondary upper lip—in certain instances oral to the two nasal apertures and in others passing between them—depends upon the position, at the time the fold of this lip pushes forward, of the nasal apertures relative to the upper edge of the mouth, and also upon the height of the fold and the length of the gape of the mouth. Where the gape is short and the fold of the lip is high, as in *Heterodontus*, the fold naturally passes between the two apertures. The extent of the cranial flexure at the time of the formation of the fold may also have some influence on its relations to the nasal apertures.

The importance and wide distribution of the labial and supra-maxillary folds would seem to indicate that the furrows related to those folds can not be simple adventitious creases in the external dermis, and the evident inference is that they may represent persisting remnants of a premandibular cleft or clefts. This, if so, would not affect any of the conclusions I have arrived at, for the related arch or arches would still necessarily lie morphologically posterior to the oral plate of embryos. The mouth could not, however, in that case, be developed from the mandibular branchial clefts fused with each other in the mid-ventral line, for the edge of mouth lies anterior to all the labial folds and furrows. The mouth would then, of necessity, be a terminal opening formed by the breaking through of the anterior wall of the gut, that wall being represented in the oral plate of embryos.

In the Cyclostomata the upper lip lies between the hypophysis and the oral plate, and it is highly probable that it represents the primary lip of all vertebrates. If this be so, the lips in these fishes are, as compared with those in other vertebrates, primitive and not degenerate structures. His calls this lip the 'Schnauzenfalte,' but it is certainly not the 'Schnauzenfalte' of his descriptions of the Mammalia. The supramaxillary fold of the Holocephali and Dipneusti is perhaps represented in the

slight fold shown projecting ventro-anteriorly dorsal to the nasal epithelium in His's figure of a median sagittal section of *Ammocoetes*.

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Dec. 1, 1916.

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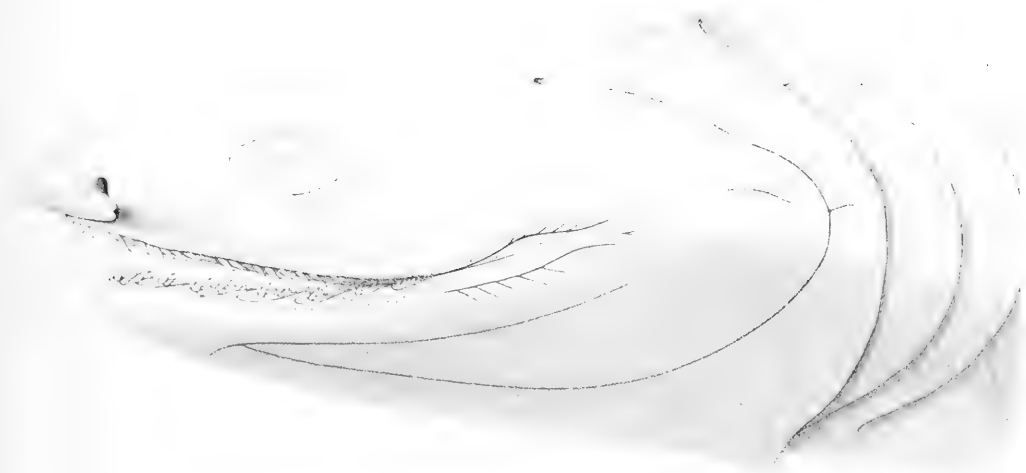
PLATE 1

EXPLANATION OF FIGURES

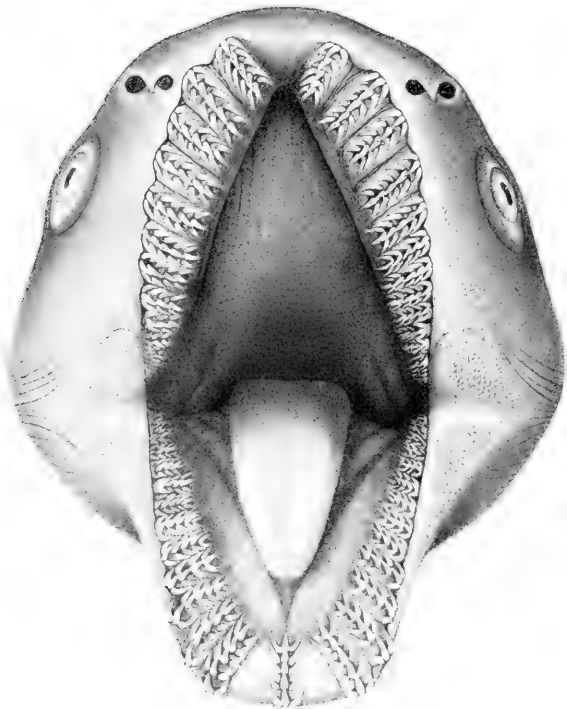
- 1 Lateral view of head of *Chlamydoselachus anguineus*. $\times \frac{2}{3}$.
- 2 Front view of same, with mouth forced widely open. $\times \frac{2}{3}$.

ABBREVIATIONS

<i>a'a</i> , antero-lateral nasal aperture	<i>pll</i> , primary lower lip
<i>ama</i> , antero-mesial nasal aperture	<i>pma</i> , postero-mesial nasal aperture
<i>a</i> process <i>a</i> of ala nasalis	<i>pml</i> , premaxillary lip
<i>a'</i> process <i>a'</i> of ala nasalis	<i>pul</i> , primary upper lip
<i>β</i> process of <i>β</i> of ala nasalis	<i>sag</i> , secondary angle of the gape
<i>lag</i> , line of angle of gape	<i>sll</i> , secondary lower lip
<i>lfd</i> , labial fold	<i>smf</i> , supramandibular furrow
<i>mple</i> , mandibular preangular labial crease	<i>smfd</i> , supramandibular fold
<i>nbg</i> , naso-buccal groove	<i>smxfd</i> , supramaxillary fold
<i>nfd</i> , nasal fold	<i>ssll</i> , supplementary secondary lower lip
<i>nfl</i> , nasal flap	<i>ssul</i> , supplementary secondary upper lip
<i>nfr</i> , nasal frill	<i>lip</i>
<i>nlfd</i> , naso-labial fold	<i>sul</i> , secondary upper lip
<i>pag</i> , primary angle of gape	<i>tag</i> , tertiary angle of the gape
<i>pla</i> , postero-lateral nasal aperture	<i>tul</i> , tertiary upper lip
	<i>vp</i> , valvular process



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PLATE 2

EXPLANATION OF FIGURES

- 3 Ventral view of head of *Pristis antiochorum*. Copied from Müller and Henle.
- 4 Ventral view of head of *Scyllium canicula*. $\times 2$.
- 5 Ventral view of head of *Chiloscyllium punctatum*. Copied from Müller and Henle.
- 6 Ventral view of head of *Heterodontus francisci*. $\times 2$.
- 7 The same, the lower jaw removed and the nasal frill pulled back on left hand side of figure. $\times 2$.



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PLATE 3

EXPLANATION OF FIGURES

- 8 Ventral view of head of *Mustelus* (probably *vulgaris*). $\times 1$.
- 9 The same, dissected so as to show the ala nasalis on left hand side of figure, and the nasal pit on right hand side. $\times 1$.
- 10 Ventral (external) view of left ala nasalis of *Mustelus*. $\times 2$.
- 11 Dorsal (internal) view of the same. $\times 2$.
- 12 Lateral view of the head of *Ceratodus forsteri*. $\times 1$.

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PLATE 4

EXPLANATION OF FIGURES

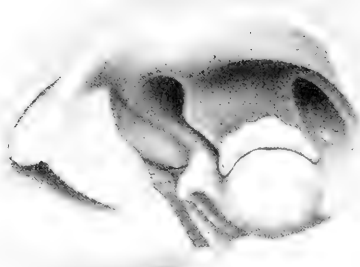
- 13 Lateral view of the head of *Chimaera colliei*. $\times 1$.
- 14 *Chimaera colliei*; view perpendicular to the ventral surface of the snout, naso-labial fold turned back on left hand side of figure.
- 15 *Chimaera colliei*; lateral view of the nasal region, the naso-labial fold turned back. $\times 2$.
- 16 The same; the valvular process also turned back. $\times 2$.



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THE MYODOME AND TRIGEMINO-FACIALIS CHAMBER OF FISHES AND THE CORRESPONDING CAVITIES IN HIGHER VERTEBRATES

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A functional myodome, or so-called eye-muscle canal, is a structure peculiar to fishes, and even among them it is limited, in the fishes I have examined to *Amia* and the non-siluroid Teleosts. It is such an important organ in those fishes in which it is found that it has necessarily received considerable attention, and various suggestions have been made regarding its origin and development. In my work on the Mail-cheeked Fishes (Allis, '09) it was discussed at considerable length, and I came to the conclusion that it was primarily a subpituitary and intramural space which had been secondarily invaded by certain of the rectus muscles, entrance to it having been acquired, on either side, through a foramen that transmitted a cross-commissural vein which drained the pituitary region and more particularly the hypophysis. That any part of the definitive myodome formed part of the *cavum cerebrale cranii*, that any part of it had been excavated by certain of the rectus muscles in previously solid portions of the *basis cranii*, simply in order to acquire more favorable points of origin, or that any part of it had been enclosed by the growth of bone or cartilage developed for that special purpose, I did not believe. I accordingly did not, at the time my manuscript was sent to press, accept Swinnerton's ('02) contention that, in *Gasterosteus*, the anterior portion of the myodome was an actual derivative of the *cavum cerebrale cranii*, while its posterior portion was an extramural space secondarily enclosed between the basioccipital and the underlying *parsphenoid*. I, however, later received Gaupp's ('05 b) work on *Salmo*, and when I found that he had arrived

at practically the same conclusion as Swinnerton, I added the following foot-note to my own work (Allis, '09, p. 195):

Gaupp, in Bd. 3 of Hertwig's *Handbuch der vergleichenden und experimentellen Entwicklungslehre der Wirbeltiere*, a work that I have only seen since this manuscript was sent to press, describes practically similar conditions in *Salmo* [to those described by Swinnerton in *Gasterosteus*], and arrives at practically similar conclusions regarding the homologies of the parts. This would seem to establish the fact that the basioccipital portion of the myodome is extracranial in origin. Regarding the prootic portion of the myodome, Gaupp's descriptions would seem to confirm my contention that it is an intramural space and not an intracranial one.

According to the views set forth in the works above referred to, both Swinnerton and Gaupp maintain that the myodome owes its origin to the fact that certain of the muscles of the eyeball, which primarily had their points of origin on the external surface of the chondrocranium, forced their way into the *cavum cerebrale cranii*, forced the brain upward considerably above the *basis cranii*, and then, after having thus displaced and certainly disturbed the delicate central nervous organ, forced their way out of the chondrocranium to again acquire origin on its external surface, and then became secondarily enclosed there in a canal developed for that special purpose. This has always seemed to me improbable, notwithstanding my provisional and somewhat qualified acceptance of it, and I have long intended investigating the development of this canal whenever I could obtain suitable material, my series of sections of somewhat advanced teleostean embryos not being considered suitable for the purpose.

I, however, recently had occasion, in connection with other work, to examine a series of sections of a 51-mm. specimen of *Hyodon tergisus*, and noticed that the myodome was directly continuous, posteriorly, with a groove on the ventral surface of the basioccipital that lodged the anterior portion of the median dorsal aorta. This at once suggested that the basioccipital portion of the telostean myodome, and hence possibly the entire myodome, might be a canal of vertebral origin comparable to the haemal canal of the tail, for that canal is not

always limited to the caudal region, as the conditions in *Acipenser* show (Bridge, '04, fig. 115, p. 200).

Accordingly, with this idea definitely in view, I have carefully examined the myodome, not only in this series of sections of *Hyodon* and in a single prepared skull that I have of the adult fish, but also in all other embryos and adults of the *Holostei* and *Teleostei* that I at present have at my disposal that seem to be of interest in this connection, and the result has been not only to strongly favor this interpretation of the conditions, but also to give a conception of the myodome itself and the bones related to it quite different from that I formerly held. The functional myodome, as found in the fishes examined, will first be quite fully described, and then comparison made with the descriptions of the corresponding parts in certain other fishes and in certain of the higher vertebrates.

The preliminary examination of the serial sections used in connection with the work was wholly done by my assistant, Mr. John Henry, camera drawings being made of many sections of each series. The drawings used for the figures are by my assistant, Mr. Jujira Nomura.

HYODON TERGISUS

The myodome of *Hyodon* has never been described, so far as I can find, except by Ridewood, ('04), who only says that, in *Hyodon alosoides*: "The parasphenoid underlies but a small portion of the basioccipital, and the eye-muscle canal opens at its posterior end by an oval foramen."

In my skull of the adult *Hyodon tergisus*, the anterior opening of the myodome is triangular and unusually large and tall for the size of the skull. Posterior to this opening the myodome diminishes rapidly in size, and finally becomes continuous with an open groove on the ventral surface of the basioccipital. This groove extends to the hind end of the basioccipital and there cuts through the ventral edge of the vertebra-like hind end of the bone to open upon its posterior surface. This open groove forms no part of the myodome as described by Ridewood, his myodome ending at the oval foramen described by him, which leads from

the myodome directly into the anterior end of the groove. The groove quite unquestionably lodged, in the fresh specimen, the anterior portion of the dorsal aorta, as it does in my 51-mm. specimen, and it may accordingly be called the aortal groove, the term myodome being limited to that canal as described by Ridewood.

The anterior portion of the floor of the myodome, as thus defined and limited, is formed by the parasphenoid, the ascending processes of which form the ventral portions of its side walls. The middle portion of the floor is probably formed by the synchondrosis, in the mid-ventral line, dorsal to the parasphenoid, of the ventral ends of the ventral processes of the prootics, for that is the condition in my 51-mm. specimen, but, as I do not wish to destroy my one skull of the adult of this fish, I cannot definitely say that this is so. A short posterior portion of the floor is probably formed, like its anterior portion, by the parasphenoid, for it is so formed in the 51-mm. specimen. Slightly posterior to the sutural line between the hind edges of the prootics and the anterior end of the basioccipital, the parasphenoid separates into two diverging hind ends which extend posteriorly a certain distance, there resting upon the ventral edges of the bounding walls of the aortal groove.

The dorsal portions of the side walls of the anterior portion of the myodome and the entire side walls of its posterior portion are formed by the ventral processes of the prootics, which are overlapped externally by the lateral edges of the parasphenoid. The roof of the myodome is formed by the horizontal processes of the prootics, the so-called prootic bridge or shelf, but whether these processes suture with each other in the median line or are separated by a median line of cartilage, I cannot tell from my specimen for the reason above given. The prootic bridge is perforated near its anterior edge by a small median foramen, the so-called pituitary opening of the brain case of my descriptions of other fishes, the bridge thus having post-pituitary and prepituitary portions.

The basisphenoid, which, as Ridewood says, has no vertically descending process, suturates posteriorly with the anterior edge of the prepituitary portion of the prootic bridge; later-

ally, on either side, with the related alisphenoid, and anteriorly with the orbitosphenoid. It is perforated by a single median foramen which transmits the two optic nerves, this apparently agreeing with Ridewood's description of this bone, for his statement that it "forms the superior edge of the optic foramen" must mean that the two optic nerves traverse it through a single opening.

As already stated, the groove on the ventral surface of the basioccipital of my 51-mm. specimen lodges the anterior portion of the median dorsal aorta. When, proceeding anteriorly, the aorta begins to widen, preparatory to separating into a lateral dorsal aorta on either side, it recedes from the groove and is replaced by the hind ends of the muscoli recti externi; these muscles soon occupying the entire groove, the aorta lying ventral to them and outside the groove. The lateral edges of the groove give insertion to the tunica externa of the air-bladder, the tissues of the tunica forming, in the posterior, but not the anterior portion of the groove, an arched bridge beneath the aorta and so enclosing it in a canal; this being as described by Bridge ('99) in *Notopterus*. The notochord, enclosed in the basioccipital, lies directly above the bottom of the groove, separated from it by only a thin layer of bone of perichordal origin.

In sections through the extreme hind end of the basioccipital (fig. 12) the aortal groove is shallow and lies directly beneath the notochord, between blocks of cartilage which are unquestionably the homologues of the lower arches, or basiventrals, of current descriptions of the vertebrae, but which I shall refer to as the ventrolateral vertebral processes. On each of these processes there are two slight ridges: a ventromesial one, clothed with perichondrial bone that represents a part of the hind end of the basioccipital, and a dorsolateral one, not clothed with bone, which gives attachment to a ligament running outward in an intermuscular septum and doubtless representing either a dorsal or a ventral rib. In sections slightly farther forward (fig. 11) the ventrolateral cartilaginous processes have entirely disappeared, but are represented by parts of the basioccipital which

are of membrane origin. The dorsolateral ridge of the process of the preceding figure has disappeared, but the ventromesial one is represented by a tall ridge of bone which bounds laterally on either side a deep aortal groove. The dorsolateral vertebral processes (upper arches, basidorsals) are here represented by two large blocks of cartilage in relation to which the exoccipitals are developed.

Each exoccipital is perforated by two occipital nerves, the anterior one represented by a ventral root alone and the posterior one by both dorsal and ventral roots. Anterior to these two nerves a delicate ventral root arises from the medulla, but it does not reach the internal surface of the cranial wall.

On the ventral surface of the first free vertebra there is a slight depression, but no aortal groove, the space between the ventrolateral vertebral processes of opposite sides being completely filled by bony deposits.

Comparing these conditions with those in the adult *Amia*, it is evident that the ventromesial ridges of the ventrolateral cartilaginous processes of *Hyodon*, bounding laterally the aortal groove, are the homologues of the little cartilaginous processes on the ventral surface of the hind end of the basioccipital of *Amia*. There are in *Amia*, as is well known, two pairs of these little cartilaginous processes—called by Hay ('95) aortal supports, by Schauinsland ('05) haemal processes—and Schauinsland says that they are related to certain vertebrae that were said by Sagemehl ('83) to have fused with the hind end of the primordial cranium of this fish. Schauinsland further says that Sagemehl found one pair of these processes, but that he himself finds two; but these two pairs had already been described by both me ('97) and Schreiner ('02). The space between each pair of these processes is almost completely filled by bony deposit, the aortal groove thus being obliterated here, as it is also shown by Hay (95, fig. 1) to be in a transverse section of one of the anterior dorsal (trunk) vertebrae of a 12.5-cm. specimen of this fish. In the last dorsal vertebra of this same specimen, Hay shows (*l.c.*, fig. 6) this space not so completely filled by bony deposit, and in the first caudal vertebra it is even still less so

(*l. c.*, fig. 7). If, in those vertebrae that are known to have fused with the occipital region of the cranium of this fish, the deposit of bone between the aortal supports had been as restricted as it is in the first caudal vertebra, conditions similar to those actually found in *Hyodon* would have arisen. The posterior portion of the aortal groove of *Hyodon* is thus certainly enclosed between processes of vertebral origin, but whether these processes are the exact homologues of the haemal processes of the tail is open to some question, for there is marked want of accord in the descriptions of the formation of the latter.

In *Amia* the aortal supports (haemal processes, Schauinsland) are said by both Hay and Schauinsland to be primarily cartilaginous, and to be simply differentiated parts of the bases of ventrolateral vertebral processes (lower arches). Posterior to the twenty-fourth vertebra, these supports are said by Hay to be developed apparently independently of the main mass of the ventrolateral vertebral processes (lower arches), and in the posterior region of the trunk they are said to be forced away from the notochord by bony deposits, and to each there become attached to the ventral surface of the remaining portion of the related ventrolateral process, which is then called a parapophysis. In the tail region the aortal supports are said by Hay to entirely disappear, and this one statement, together with the several figures given, would lead one to suppose that it is the remaining portions only of the ventrolateral processes, the so-called parapophyses, that form the haemal arches of the tail. The descriptions are, however, not clear as to this. What Hay actually says is ('95, p. 16):

In the vertebrae of the tail the cartilages [aortal supports] are missing. There is, however, in my younger specimen, what seems to be vestiges of them in the first caudal vertebra. Nothing, however, can be more certain than that the lower arches of the trunk are bent down to form the arches of the tail, and that the aortal supports have nothing to do with the formation of the caudal haemal arches.

On a later page he, however, says: "In the tail the halves of each lower arch have united at their distal ends, so as to enclose the blood vessels." It may accordingly be that Hay con-

sidered the haemal arches to be formed by the entire ventrolateral processes, and this is what Schauinsland ('05) says of these arches in all fishes. In *Laemargus*, Schauinsland even shows (*l. c.*, p. 411) the aortal supports (his haemal arches) projecting mesially from the mesial surfaces of the entire ventrolateral processes and partly separating the haemal canal into dorsal and ventral compartments which lodge, respectively, the aorta and the caudal vein.

In *Polypterus* the haemal arches have, as described by Budgett ('02), a totally different origin from that above set forth. In a 30-mm. specimen of this fish Budgett finds three distinctly separate series of cartilaginous vertebral processes, one dorsal, one lateral, and the other ventral. The lateral processes bear the upper ribs, which have the positions of the ribs in the *Selachii*. The ventral processes bear the lower ribs, which have the position of the ribs in the *Teleostei*. It is said that, in the caudal region, "the lateral series of cartilages are not found, while the ventral cartilages, though retaining their position, become the greatly enlarged haemal arches." These latter arches are thus here formed by processes that are certainly not the homologues of the so-called lower arches of the *Selachii*. In the trunk region of specimens of *Polypterus* older than the 30-mm. one, the ventral processes are said to be forced away from the notochord by bony deposits formed in relation to the lateral processes and these bony deposits are shown, in Budgett's figures, forming so-called aortal supports on either side of the aorta. The ventral processes thus forced away from the notochord are then found as blocks of cartilage in the bases of the ventral ribs at some distance from the notochord and loosely attached to the under sides of the lateral processes; these ventral processes of this fish thus strikingly resembling the aortal supports of Hay's descriptions of *Amia*.

The aortal supports and haemal arches, as those terms are employed by English authors, may thus be of different origin in different fishes, but, whatever their origins and homologies may be, the lateral walls of the aortal groove of *Hyodon*, in the posterior basioccipital region here under consideration, are quite

certainly formed by aortal supports, the remainder of each primitive ventrolateral vertebral process being represented in the little cartilaginous ridge that gives attachment to the ligament which runs outward, rib-like, in the related intermuscular septum.

Returning now to the descriptions of Hyodon and proceeding forward in the sections, figure 10 shows that a ridge of bone gradually appears lateral to the tall ridge that bounds on either side the aortal groove, the appearance in these sections somewhat suggesting, excepting in the absence of cartilage, the conditions shown in Hay's figures 5 and 6 of trunk vertebrae of *Amia*, where the aortal supports are attached to the ventromesial surfaces of the lower arches (parapophyses, Hay). Still farther forward, in sections through the hind ends of the parasphenoid (fig. 9), the mesial one of these two processes has disappeared while the lateral one persists as a stout low process, this giving a broad ventral edge to the aortal groove. The lateral walls of the aortal groove are now formed by the entire ventrolateral processes, and not simply by the aortal supports, and the bony deposits on either side that fill the space between these processes and the dorsolateral vertebral processes (upper arches) has been excavated to form the recessus sacculi. Hence the basioccipital is here W-shaped in transverse section, the two grooves on the dorsal surface of this W each forming the ventral portion of the related recessus sacculi, and the grooves of opposite sides being separated from each other by a tall median plate formed by part of the basioccipital. The notochord lies in the ventral end of this median plate, dorsal to the bottom of the aortal groove, but is here represented simply by a notochordal space. The exoccipital of either side has vertical and horizontal plates, the former forming the dorsal portion of the side wall of the related recessus sacculi and the mesial portion of the other the roof of the recessus, the mesial edge of the latter plate resting upon the dorsal end of the tall median plate of the basioccipital and forming, with its fellow of the opposite side, the floor of the *cavum cerebrale cranii*. The median groove on the ventral surface of the W here lodges the hind ends of the

musculi recti externi, with the dorsal aorta lying ventral to them.

Proceeding anteriorly from this point to sections through the bases of the diverging hind ends of the parasphenoid (fig. 8), the bony bounding walls of the aortal groove are gradually replaced by cartilage lined with thin plates of perichondrial bone which form parts of the basioccipital. Angles in this cartilage and bone now replace the two bony ridges, just described, in more posterior sections. The perichondrial bone then disappears, in the region of the hind end of the myodome, leaving the bounding walls of the groove entirely of cartilage, and slightly anterior to that point the remaining portions of the basioccipital also vanish. The notochord extends forward nearly to the hind end of the myodome, its anterior end lying dorsal to the bottom of the aortal groove and hence in the level of the roof of the myodome and not in that of its floor. In this region the aorta has separated into a lateral dorsal aorta on either side.

Anterior to the bases of the diverging hind ends of the parasphenoid, the aortal groove is closed ventrally by the latter bone, and, still lodging the musculi recti externi, becomes the hind end of the myodome. Except that the groove is here closed ventrally by the parasphenoid and that it lies in the protic region, there is no line of demarcation between it and the open canal in the basioccipital region, and each broad ventral edge of the open groove, lying between the two angles above described, is continued forward as the ventral edge of the lateral bounding wall of the myodomic canal.

Proceeding forward in the sections, there is gradual ventral growth of the cartilaginous side walls of the myodomic canal, this growth taking place between the two little angles above described. This gives rise to a flange of cartilage on either edge of the primitive groove, the flange projecting ventrally and slightly mesially beneath the level of the dorsolaterally projecting basal portion of the lateral wall of the cranium, the base of that portion of the wall lying in the level of the ventral edge of the primitive aortal groove (fig. 7). Proceeding anteriorly, these flanges increase gradually in actual height, and appear to

gain additional height because of the gradual widening of the dorsal portion of the myodomic cavity, cross-sections through which change gradually from oval to pear-shaped and then to triangular. The roof of the myodome thus becomes flat (fig. 6,) instead of being arched (fig. 7). The cartilage forming the top of the arched roof is continued forward as the median portion of the flat roof, and is enclosed between plates of perichondrial bone which do not meet in the median line and which form the distal (mesial) portions of the horizontal processes of the prootics. The lateral portions of these processes and the dorsal portions of the side walls of the myodome are now each formed by two plates of bone, doubtless of perichondrial origin but without enclosed cartilage, this bone replacing the cartilage of the preceding sections and forming the dorsolateral corners of the myodomic cavity. The horizontal portion of each of these angles of bone forms the basal (lateral) portion of the horizontal process of the prootic of its side, and arises from the base of the lateral wall of the *cavum cerebrale cranii*. The vertical portion of the angle of bone forms a wall between the dorsal portion of the myodome and the ventral portion of what is, in the prepared skull of the adult, a large bay on the external surface of the cranium. This bay forms that part of the large auditory fenestra of Ridewood's ('04) descriptions that lies anterior to the so-called vertical lamina of the prootic, and its floor is formed, in my embryo as in the adult, by a laterally projecting, horizontal shelf of the prootic. This bay of this fish corresponds to the facialis part of the trigemino-facialis chamber of my description of *Scomber* and the mail-cheeked fishes, and occupies a position, relative to the cranial walls, similar to that of the recessus sacculi, the floor of the bay being an anterior continuation of that of the recessus. The *truncus hyomandibularis facialis* enters this bay through a foramen in its mesial, cranial wall, and runs outward above its floor. The *vena jugularis*, traced from behind forward, enters the bay over the posterior edge of its floor, accompanied by a sympathetic nerve, a communicating branch from the *nervus glosso-pharyngeus* to the *nervus facialis*, and the *arteria carotis externa*, this artery lying ventromesial to the other structures.

The foramen faciale perforates the prootic posterior to the postorbital process of the neurocranium, the foramen trigeminum perforating the same bone anterior to that process, the two foramina both leading directly into the cavum cerebrale cranii. Between these two foramina the postorbital process of the cranium is perforated by a short canal, the floor of which lies at the level of the roof of the myodome and hence dorsal to the posterior portion of the floor of the facialis bay. The later bay leads directly into this canal, the canal itself leading into the orbit and transmitting the vena jugularis, the arteria carotis externa, a communicating branch from the nervus facialis to the nervus trigeminus, and a sympathetic nerve. This canal is thus a jugular canal through the prootic bone, and it represents all there is, in this fish, of the trigemino-facialis chamber of my description of others of the Teleostei. There are in this region of the cranium of fishes three distinctly different chambers. One is the trigemino-facialis recess of my descriptions of the Teleostei and Selachii; another is the jugular canal through the prootic, just referred to and which I have heretofore called the teleostean trigemino-facialis chamber; and the third is a chamber formed by the fusion of the other two, and is the trigemino-facialis chamber of my descriptions of *Amia*. It is accordingly necessary to distinguish between these several chambers, and the term trigemino-facialis chamber will hereafter be limited to the chamber as found in *Amia*, the two parts of the chamber being called its *pars ganglionaris* and *pars jugularis*.

The ventral edges of the side walls of the myodome of *Hypodon* are nowhere enclosed in perichondrial bone, cartilage always projecting ventrally beyond the related bone and abutting against dense connective tissue that separates it from the parasphenoid. This tissue is apparently all skeletogenous, for there is no definite perichondrial membrane separating it from the cartilage. The parasphenoid develops in the outer layers of this tissue, and the bases of the diverging hind ends of that bone are connected by it across the median line (figs. 8 and 9), the tissue there forming a dense and well-defined band-like layer. Farther posteriorly this transverse band becomes less dense,

and then practically disappears, but there is always a line connecting the two ends of the parasphenoid, and hence also connecting the ventral edges of the aortal groove.

Approximately in the transverse plane of the foramen faciale, a cartilaginous process projects mesially from the ventral end of each cartilaginous side wall of the myodome and meets its fellow of the opposite side in the median line, but it does not completely fuse with it, a slight line of separation always remaining evident. These two processes thus together form a cartilaginous floor to the myodome, the parasphenoid lying against the ventral surface of this floor, but separated from it by the dense skeletogenous tissue above referred to. Immediately posterior to this point, the pharyngobranchial of the first branchial arch articulates with the dorsal portion of the side wall of the myodome, there lying between the vena jugularis and the external and internal carotid arteries; and immediately posterior to that, the pharyngobranchial of the second branchial arch articulates with the ventral surface of the parasphenoid (fig. 7). The external and internal carotid arteries separate from each other slightly anterior to the latter point, both lying along the lateral surface of the lateral wall of the myodome. The external carotid runs forward and upward, ventral to the nervus facialis, and, joining the vena jugularis, traverses, with that vein, the short canal which represents the pars jugularis of the trigemino-facialis chamber. The internal carotid continues forward and downward along the side wall of the myodome until it reaches the hind edge of the ascending process of the parasphenoid, where it traverses a foramen which is, as in the adult, entirely enclosed in that bone.

Beginning immediately posterior to this foramen (fig. 5) for the internal carotid artery and proceeding forward in the sections, the cartilage forming the floor of the myodome, and also the ventral ends of its side walls, gradually disappears and is replaced by the dense skeletogenous tissue already referred to several times, and in it a cavity appears, bounded on all sides by the tissue and lying between the parasphenoid and the myodomie cavity. The floor and side walls of this cavity

form a matrix, in relation to which the body and ascending processes of the parasphenoid are developed, and from here forward for a certain distance teeth are found developed in relation to this bone. The roof of the cavity forms a membrane which extends transversely from the ventral end of one persisting cartilaginous side wall of the myodome to the other, this membrane being horizontal in position in its posterior portion, but arching upward anteriorly to such an extent that, in the subpituitary region, its summit reaches nearly to the middle of the height of the entire myodomic cavity. The parasphenoid has in this region been inclining quite rapidly ventrally, this, and the arching upward of the membrane, leaving a space between the two and separating the myodomic cavity into dorsal and ventral compartments. The ventral compartment, limited to the region of the ascending processes of the parasphenoid, is bounded both laterally and ventrally by that bone. The dorsal portion of the dorsal compartment is bounded laterally by the ventral processes of the prootic bone, its ventral portion being bounded in part by the ventral portions of those processes, overlapped externally by the ascending processes of the parasphenoid, and in part by the latter processes only. The dorsal compartment still lodges the recti externi, the ventral compartment lodging the hind ends of the recti interni and the internal carotid arteries, the two being separated by a delicate line of tissue (fig. 3).

Slightly posterior to the internal carotid foramina in the parasphenoid, the roof of the myodome, formed by the horizontal processes of the prootics, is traversed at each lateral edge by both the nervus abducens and the ramus palatinus facialis, apparently through a single foramen (fig. 4). The abducens goes directly to the mucus rectus externus. The palatinus facialis runs ventrally along the internal surface of the side wall of the dorsal compartment of the myodome, passes through a notch in the anterior edge of the ventral end of the prootic portion of that wall, which is wholly of cartilage, and then, continuing ventrally between that cartilaginous wall and the ascending process of the parasphenoid, enters that portion of the ventral

compartment of the myodome that is occupied by the internal carotids (fig. 3). In its passage along the mesial surface of the lateral wall of the myodome it lies between the wall and a delicate layer of connective tissue which everywhere lines the myodomic cavity, thus apparently not definitely entering the central cavity of the myodome.

Slightly anterior to the point where the prootic bridge is perforated by the *nervi abducens* and *palatinus facialis*, and slightly anterior also to the transverse plane of the internal carotid foramina, the median cartilaginous portion of the prootic bridge ceases, and the roof of the myodome is then perforated by what is, in the prepared cranium of the adult fish, the pituitary opening of the brain case (fig. 1 to 3). This opening is closed, in fresh specimens by a portion of the *dura mater* that projects ventrally into the myodome and so forms a pit-like depression in the floor of the *cavum cerebrale cranii*, in which the pituitary body lies; thus forming the actual pituitary fossa. It will, however, be best to call it the pituitary sac, for the term pituitary fossa, and its equivalent *sella turcica*, has been given to the depression that, in the floor of the cartilaginous or osseous cranial cavity, lodges this pituitary sac, and the two are not always coincident. The sac forms the roof of this part of the myodome of *Hyodon*, and a median vertical membrane descends from its ventral and anteroventral surfaces. Anteriorly this membrane is directly continuous with the membranous interorbital septum; posteroventrally it is continuous with the anterior edge of the median portion of the horizontal myodomic membrane, the lateral portions of the latter membrane here being so broken up and interrupted by the muscles and vessels entering or leaving the myodome that they cannot be followed in the sections. The vertical membrane does not at this point extend ventrally to the floor of the myodome, but in the transverse plane of the hind edge of the basisphenoid (fig. 1) it becomes the interorbital septum, and there its flaring ventral edges are each attached to a ridge on the related lateral edge of the dorsal surface of the parasphenoid. In the triangular space enclosed between the latter bone and the V-shaped ventral end of the septum lies,

on either side, the related *nervus palatinus facialis*, the nerves of opposite sides being separated from each other by a median ridge on the dorsal surface of the parasphenoid, and each accompanied by a small branch of the internal carotid, given off just before that artery enters its foramen in the parasphenoid. This branch does not enter the myodome, but runs forward in a canal in the parasphenoid between what seem to be portions of the bone that are the one of membrane and the other of dental origin.

The rectus inferior muscle of either side has its origin on the dorsal portion of the interorbital septum, near the level of the posterior edge of the basisphenoid. The rectus superior has its origin on the anterior edge of the horizontal myodomic membrane. The pituitary vein of either side enters the dorsal compartment of the myodome in the subpituitary region, and, running posteriorly in it, joins its fellow of the opposite side posterior to the membranous pituitary sac, there forming a large sinus. From this sinus branches are sent to the rectus externus muscles, and from its anterior end a small median branch is sent upward, through the membranous roof of the myodome, into the *cavum cerebrale cranii*, where it immediately breaks up and cannot be followed in the sections.

The pituitary vein of either side is joined by veins from the eyeball and the eye-muscles, these together forming what Allen ('05) has called the internal jugular vein. I have also employed his term in certain of my works, in others calling it simply the jugular vein. This latter term is certainly the only one that can be appropriately employed, for the vein is the definitive *vena jugularis*, and as it is formed in part by the *vena capitis media* and in part by the *vena capitis lateralis*, neither of these terms can be employed excepting to designate certain sections of it.

The internal carotid artery of either side traverses its foramen at the hind edge of the ascending process of the parasphenoid and enters the ventral portion of the ventral compartment of the myodome. There it gives off the orbitonasal artery and then, running forward into the subpituitary portion of the myodome, turns upward in that part of the median vertical myodomic

membrane which forms the anterior wall of the membranous pituitary sac. In this part of its course, and while still enclosed in the median vertical membrane, it anastomoses with its fellow of the opposite side, and then, separating from its fellow, enters the *cavum cerebrale cranii* (fig. 2). There it immediately divides into anterior and posterior divisions. The posterior division sends branches to the hypophysis, and then itself separates into anterior and posterior branches. The anterior branch runs forward along the floor of the *cavum cerebrale cranii* and sends a branch outward in the body of the optic nerve. Other branches are sent to the brain, one of them joining, anterior to the *nervus opticus*, the terminal portion of the anterior division of the entire artery. In one of two specimens examined, the latter division of the artery immediately issued from the cranial cavity by passing ventrally across the posterior edge of the basisphenoid, while in the other specimen it perforated that bone near its hind edge. In each case the artery then ran forward ventral to the horizontal plate of the basisphenoid, enclosed in the dense fibrous tissues that there form the dorsal edge of the interorbital septum. While in this tissue a branch is sent outward to the eyeball, the artery then issuing from the fibrous tissue, passing across the posterodorsal surface of the *nervus opticus*, and entering the cranial cavity through the foramen for that nerve. There it joins and fuses with the anterior branch of the posterior division of the entire artery, just described, the artery so formed then running forward along the floor of the *cavum cerebrale cranii*. The branch sent to the eyeball from the anterior division of the artery, enters it close to the point of entrance of the *nervus opticus*, and there immediately forms a slight enlargement which somewhat resembles a glomus. From this glomus a branch arises and unites with the small artery accompanying the *nervus opticus*, the two together forming the *arteria centralis retinae*.

I cannot recognize the anterior division of the internal carotid artery, above described, in any descriptions that I have of the adults of fishes, and yet it is found in all of the non-siluroid Teleostei that I have examined in serial sections in connection

with the present work. The fact that, in one of my two specimens of *Hyodon*, it perforates the basisphenoid, is peculiar, and it is to be noted that in that specimen this bone has a greater anteroposterior extent than in the other, extending posteriorly beyond the sutural line between the alisphenoid and prootic, instead of ending anterior to that line, as in the other specimen. This, when compared with the conditions in the other fishes examined, to be described later, would seem to indicate that the basisphenoid of *Hyodon* is not strictly comparable to that bone in those other fishes.

On one side of my 51-mm. specimen of *Hyodon* the efferent pseudobranchial artery entered the ventral compartment of the myodome with the internal carotid, through the foramen for that artery. On the other side it perforated the ascending process of the parasphenoid anterior to the internal carotid, separated from it by a narrow column of bone. Having entered the ventral compartment of the myodome, in its subpituitary portion, it passes ventral to the orbitonasal artery and is connected with its fellow of the opposite side by a cross-commissural vessel which passes anteroventral to the internal carotids. The efferent pseudobranchial artery then itself runs outward into the orbit, as the *arteria ophtalmica magna*, to enter the eyeball and there supply the chorioid gland.

In this 51-mm. embryo, as in the adult, the alisphenoid bone has no pedicel (parasphenoid leg), this pedicel being represented by membrane only, as it is, wholly or in part, in many other Teleostei (Allis, '09). The pedicel or so-called vertical descending process (Ridewood) of the basisphenoid is also wanting, as already stated, that bone being represented by its horizontal plate alone. In those Teleosts in which this bone has a pedicel, its hind edge forms the median vertical anterior boundary of the myodome, and the anterior edge of the median vertical myodomic membrane is attached to it. When the pedicel is wanting, as in *Hyodon*, the vertical myodomic membrane runs insensibly into the membranous interorbital septum, and there is nothing to mark definitely its anterior limit.

The myodome of my 51-mm. specimen of *Hyodon* thus lies in part beneath the pituitary opening of the brain case and in part posterior to that opening, a part of it thus being prechordal and the remainder chordal in position, or, in the terminology employed by Froriep ('02 a), the one prespinal and the other spinal. The spinal portion is formed, throughout part of its length, by two distinctly different parts, one dorsal and the other ventral, the two being completely separated from each other in part by cartilage and in part by membrane which forms a direct anterior prolongation of the cartilage. In the prespinal portion these two compartments of the spinal portion are confluent because of the breaking down of the separating wall (the horizontal myodomic membrane) by the structures that here enter or leave the dorsal compartment. The canal traversed by the internal carotid arteries as they run upward in the median vertical myodomic membrane lies in the level, anteriorly prolonged, of the dorsal myodomic compartment, but it forms no part of either compartment of the myodome.

The dorsal compartment of the myodome of *Hyodon* is limited to the subpituitary and postpituitary regions, and although both of these parts lie in the prootic region, the postpituitary portion, which lies beneath the prootic bridge, may be alone referred to as the prootic portion of the compartment. The ventral compartment has prootic, subpituitary, and prepituitary portions. The dorsal compartment is directly continuous posteriorly with the anterior end of the aortal groove, which extends the full length of the basioccipital region. The ventral compartment is not continuous with the groove and it does not extend posteriorly as far as the dorsal compartment. It lies between the floor of that compartment and the parasphenoid, lodges the hind ends of the recti interni, and is traversed by the internal carotid arteries, the palatine branches of the faciales, and the efferent pseudobranchial arteries. The dorsal compartment lodges the pituitary veins and the muscoli recti externi, these muscles entering it from the orbits and leaving it by its posterior opening. The nervus abducens of either side perforates the roof of this compartment to reach and supply the rectus

externus. The ramus palatinus facialis also perforates the roof and traverses the dorsal compartment in order to reach the ventral one, but it is separated from the central cavity of the dorsal compartment by a membrane, apparently of skeletogenous character, the nerve thus probably lying morphologically in the wall of this compartment of the myodome and not actually traversing it.

The recti externi, after issuing through the posterior opening of the dorsal compartment of the myodome, extend posteriorly a certain distance, there lying in a part of the aortal groove which differs slightly in character from the part posterior to it. This anterior part of the groove is, however, so evidently an anterior prolongation of its posterior portion that the two parts must be of similar origin, and as the posterior portion of the groove has certainly not been developed in any relation whatever to any of the muscles of the eyeball, it is certain that the anterior portion also has not been so developed. This is, furthermore, confirmed by the conditions in *Polypterus*, in which there is no functional myodome, but there is both a cavity corresponding to the dorsal compartment of the myodome of *Hyodon* and a closed and wholly separate canal lodging the cranial portion of the aorta and corresponding to the aortal groove of *Hyodon*. This myodomie cavity and aortal canal have both been referred to and discussed in certain of my earlier works (Allis, '08 a, '09), and I now find, on reexamining my sections of a small specimen of this fish, that the enclosing walls of the aortal canal give even more positive evidence of having been formed by vertebral processes than do the walls of the groove of *Hyodon*.

There thus seems little doubt that the bounding walls of the aortal groove of *Hyodon* are formed by processes similar to those which enclose the haemal canal of the tail, and that those bounding walls are formed either by the entire ventrolateral processes of vertebrae which here have been incorporated in the neurocranium, or by aortal supports developed in relation to those processes; and if the walls of this groove are so formed, it would seem as if the side walls of the prootic portion of the dorsal compartment of the myodome, evidently an anterior con-

tinuation of the walls of the aortal groove, must be of similar origin. Why the aorta has been excluded from this prootic portion of the myodome is not apparent, but it would seem as if it might be related to the development of the hypochorda. According to Stöhr ('95), the hypochorda of *Rana*, when first formed, is attached to the dorsal wall of the alimentary canal by a series of tubular bridges, which persist longer in the anterior than in the posterior region of the trunk, and, for a time, there prevent the lateral dorsal aortae from fusing with each other in the median line excepting between the bridges. In the head region the hypochorda is said to develop later than in the trunk, and the related bridges would hence there also, while they persisted, prevent the lateral dorsal aortae from fusing with each other excepting between the bridges. It may then be that, such a bridge persisting in the prootic region, the lateral dorsal aortae could not there fuse with each other, and before this bridge had disappeared they had become fixed in position by the early development of the anterior aortic arches. Anterior to the spinal region of the cranium they, however, fused with each other, in certain fishes, that point either representing an interval between two hypochordal bridges, or lying anterior to the anterior bridge, as the case may be. This would then not only explain the formation of the *circulus cephalicus*, but also account for its position external to the ventral processes of the prootics.

In further support of the assumption that the ventral processes of the prootics are formed by ventrolateral vertebral processes is the fact, possibly significant, that these processes, like the neural processes in the spinal region, enclose a large canal between their proximal portions and a smaller one between their distal ends, the two cavities being separated from each other by a horizontal partition. In my 51-mm. specimen of *Hyodon* this partition is partly of cartilage and partly of membrane. In all the other fishes examined it is wholly of membrane, excepting as that membrane may have undergone ossification as part of the parasphenoid, a median longitudinal opening thus being left, when the parasphenoid is removed, be-

tween the ventral ends of the ventral processes of the prootics. This opening is the hypophysial fenestra of Sagemehl's descriptions of *Amia* and the *Teleostei*, and I have always employed that term for it in all my works. This fenestra has, however, in a considerable part of its length, no relation whatever to the hypophysis, and it will be later shown that, in all probability, it does not even contain the so-called fenestra hypophyseos of early embryos of these fishes. The term hypophysial fenestra is thus inappropriate, and I shall hereafter refer to it as the fenestra ventralis myodomus. To facilitate the descriptions and comparisons, the ventral processes of the prootics will be considered to be ventrolateral vertebral processes, notwithstanding that this is not definitely established by my present work.

SCOMBER SCOMBER

In the adult *Scomber* I found (Allis, '03) the myodome extending nearly to the hind end of the basioccipital but not opening posteriorly; and, doubtless in direct correlation with this, the parasphenoid of this fish does not have diverging hind ends. That part of the myodome that is related to the basioccipital is enclosed between ventral flanges of that bone which closely resemble the ventral processes of the prootics and form a direct posterior continuation of them. Two membranes, one vertical and the other horizontal, were said to extend the full length of the myodome. The horizontal membrane was said to separate the myodome into dorsal and ventral parts which lodged, respectively, the recti externi and interni. The vertical membrane was said to arise from the hind edge of the pedicel of the basisphenoid and, lying between the recti interni, to bisect the ventral part of the myodome. The recti inferiores were said to arise partly from the interorbital septum, between the foramen opticum and the anterior edge of the basisphenoid, and partly from a ligament or tendon which arises from the dorsal end of the pedicel of the basisphenoid. The recti superiores were said to have their origins from the anterior edge of the horizontal membrane.

The above statements all referred only to the adult of this fish, for I at that time had no specimens small enough to be sectioned. I have, however, since prepared a series of transverse sections of a 65-mm. specimen, in which I now find the vertical membrane above referred to descending from the ventral surface of the membranous pituitary sac, as in Hyodon. That part of it which, in the adult, lies beneath the horizontal membrane and extends to the hind end of the myodome is, in this 65-mm. specimen, simply a delicate line of connective tissue, but it would nevertheless seem to represent a remnant of a wall which primarily separated this part of the myodome into two parts, one on either side, as will be later explained. The horizontal membrane is practically as I described it in the adult. In its anterior portion it is not strongly developed, and there arises, on either side, from a layer of tissue which lines the internal surface of the side wall of the myodome cavity and is continued outward around the ventral end of the wall and then upward a certain distance along its external surface. The parasphenoid rests, on either side, upon the ventral surface of this tissue, and a longitudinal ridge on either side of the dorsal surface of the bone projects upward into that part of the tissue which lines the internal surface of the side wall of the cavity; this part of the parasphenoid certainly being an ossification developed in relation to the tissue. Along the line of origin of the horizontal membrane, the cartilage of the side wall of the myodome is slightly constricted and imperfect, suggesting a segmentation line similar to that shown by Schauinsland ('05) where a rib is in process of being segmented from a lower arch in the vertebral region of certain other fishes. Near the hind end of the myodome, beginning slightly anterior to the point where the recti interni terminate, that part of the cartilage of each lateral wall of the myodome that lies ventral to this segmentation line gradually passes, without any line of demarcation, into dense fibrous tissue which forms the ventral end of each lateral wall of the myodome. The parasphenoid here lies against the ventral surface of this tissue, and the longitudinal ridge on either side of the dorsal surface of the bone extends upward along the mesial sur-

face of the tissue. The horizontal membrane is strongly developed here, and extends across the median line between the ventral ends of the persisting portions of the cartilaginous side walls, thus lying at a certain distance dorsal to the parasphenoid. Proceeding posteriorly from here, in the sections, the recti interni disappear, leaving a space between the horizontal membrane and the parasphenoid. The latter bone then shortly disappears, and the myodomic cavity is then closed ventrally by the horizontal membrane only, this condition possibly persisting to the hind end of the myodome, but my sections are here imperfect and I cannot definitely determine this.

The conditions in *Scomber* would thus arise from those in *Hyodon* if the ventral compartment of the myodome of the latter fish were extended posteriorly nearly to the hind end of the aortal groove, and the continuous myodomic-aortal cavity so formed were closed ventrally, to that point, by the parasphenoid.

The internal carotid artery of either side enters, as in *Hyodon*, the ventral compartment of the myodome, runs forward in it into the prespinal portion of the myodome, and there turns upward in the median vertical myodomic membrane, anastomosing, while in the membrane, with its fellow of the opposite side. Leaving its fellow, it separates, as in *Hyodon*, into anterior and posterior divisions both of which run upward, posterior to the basisphenoid, and enter the *cavum cerebrale cranii*. The posterior division sends branches to the hypophysis and then separates into anterior and posterior branches, the anterior branch running forward along the floor of the *cavum cerebrale cranii*, sending a branch outward with the *nervus opticus*, and then joining the anterior division of the artery, this anterior prolongation of this branch of the artery not being found in *Hyodon*. The anterior division of the artery runs forward, dorsal to the basisphenoid, and, anterior to that bone, enters the thick, dense tissues forming the floor of the *cavum cerebrale cranii* and the dorsal end of the interorbital septum, its course and distribution from there onward being as in *Hyodon*. The fact that this anterior division of the artery runs forward dorsal to the

basisphenoid would seem to show that, as already stated, the anterior portion of the basisphenoid of *Hyodon* did not primarily form part of this bone.

The cross-commissure connecting the efferent pseudobranchial arteries of opposite sides traverses the ventral part of the prespinal portion of the myodome, there passing ventral to the orbito-nasal artery and anteroventral to the internal carotids. The pituitary veins enter the dorsal compartment of the myodome and there anastomose with each other, a small branch being sent upward into the *cavum cerebrale cranii* and apparently going to the hypophysis.

The *nervus palatinus facialis* of the adult traverses a canal in the prootic which begins in the floor of the *pars jugularis* of the trigemino-facialis chamber and opens on the mesial surface of the ventral process of that bone in the plane of the hind edge of the pituitary opening of the brain case, the nerve thus apparently not traversing the dorsal compartment of the myodome. In the 65-mm. specimen the nerve does not enter the *pars jugularis* of the trigemino-facialis chamber, perforating the roof of the dorsal compartment of the myodome and traversing it, as in *Hyodon*, but, as also in *Hyodon*, there lying between the ventral process of the prootic and the lining membrane of the myodomic cavity.

MAIL-CHEEKED FISHES (LORICATI)

In the adults of *Scorpaena serofa*, *Trigla hirundo*, and *Cottus octodecimospinosus*, I found (Allis, '09) the myodome to extend nearly to the hind end of the basioccipital, and there open ventrally. In *Scorpaena* the origins of all the rectus muscles were given, the external and internal ones extending posteriorly in the myodome, the external somewhat farther than the internal. Nothing was said of a horizontal membrane separating the myodome into dorsal and ventral compartments, such as I had previously described in *Scomber* and now find in *Hyodon*. The orbital opening of the myodome was said to be closed by a strong membrane which the *recti externi* and *interni* perforated to reach their points of origin.

In a 40-mm. specimen of *Scorpaena scrofa* I now find the recti externi and interni as described in my earlier work, but they are separated by a membrane, delicate in places but well developed in others, which corresponds to the horizontal membrane of *Scomber* and *Hyodon* and separates the myodome into dorsal and ventral compartments. The rectus superior of either side arises in part from the anterior edge of this membrane and in part from the dorsal surface of the parasphenoid at or near the line where the lateral edge of the membrane is attached to it, this line being marked by a slight longitudinal ridge on the dorsal surface of the bone. The rectus inferior of either side arises from a median vertical membrane similar to that described in *Hyodon* and *Scomber*.

The internal carotid artery, after traversing its foramen at the hind edge of the ascending process of the parasphenoid, passes across an internal carotid incisure at the antero-ventral corner of the prootic cartilage, as in the adult (Allis, '09, p. 411), and enters the ventral compartment of the myodome, its farther course and distribution being as in *Scomber*. The cross-commissure of the efferent pseudobranchial arteries traverses the subpituitary portion of the myodome, as in *Hyodon* and *Scomber*. The pituitary veins anastomose with each other in the dorsal compartment of the myodome, but they do not there form an important sinus.

In the adult the ramus palatinus facialis traverses a canal in the prootic which begins in the trigemino-facialis recess (pars ganglionaris of the trigemino-facialis chamber) and opens on the internal surface of the ventral process of the prootic. In my 40-mm. embryo this nerve perforates a membranous portion of the prootic bridge, and, running ventrally between the side wall of the dorsal compartment of the myodome and the lining membrane of that cavity, as in *Hyodon* and *Scomber*, enters the ventral compartment in the subpituitary region and then escapes into the orbit.

In a 40-mm. specimen of *Trigla hirundo* the conditions are practically as in *Scorpaena*, excepting that the anterior branch of the posterior division of the internal carotid artery is inter-

rupted, either anterior or posterior to the branch sent out with the nervus opticus, as it is in *Hyodon*, while a branch, not found in either *Hyodon* or *Scorpaena*, is sent outward, anterior to the nervus opticus, to join a branch of the orbitonasal artery and then go to the eyeball.

In a 63-mm. specimen of *Trigla hirundo* the conditions differ in that the depression in the dura mater which lodges the hypophysis has a relatively wide and flat floor from which three membranes arise, one median and one at each lateral edge of the floor. These membranes are each inserted on a corresponding ridge on the dorsal surface of the parasphenoid, and the space enclosed, on either side, between them and the parasphenoid, lodges the rectus internus. Thus the ventral compartment of the myodome here rises to the ventral surface of the pituitary depression, and hence lies between right and left halves of the dorsal compartment. This condition continues posterior to the hypophysis for a certain distance, the roof of the ventral compartment of the myodome there forming the median portion of the floor of the cavum cerebrale cranii; but at the membranous anterior edge of the prootic bridge, the roof of the compartment begins to recede from the floor of the cavum cerebrale cranii, and, the lateral halves of the dorsal compartment uniting with each other above it, the conditions become as in *Scorpaena*. Apparently because of this intercalation of the ventral compartment between the anterior ends of the dorsal compartment, the pituitary veins are greatly reduced, the hypophysis being drained in part by the encephalic veins.

In this embryo of *Trigla* the nervus palatinus facialis perforates the floor of the pars jugularis of the trigemino-facialis chamber and enters the dorsal compartment of the myodome, this apparently being as I found this nerve in the adult Scomber.

In the adult *Cottus octodecimospinosus* I found (Allis, '09) the myodome continued posteriorly a short distance in the basioccipital, and not opening posteriorly on the ventral surface of the cranium. The prootics have perfectly normal horizontal processes, and they are shown, in my figures, preformed in cartilage and forming the roof of the myodome. The parasphenoid has diverging hind ends.

In a 20-mm. specimen of *Cottus scorpeus* I now find the protic portion of the myodome separated from the cavum cerebrale cranii by membrane only, no cartilaginous or osseous protic bridge being as yet developed. In its basioccipital portion the myodome lies in a groove on the ventral surface of the basioccipital (fig. 18), which opens posteriorly between the diverging hind ends of the parasphenoid, but is there closed ventrally by membrane which extends horizontally between those ends. This part of the myodome lodges the hind ends of the recti externi, the two muscles being separated from each other by a delicate vertical membrane. Proceeding anteriorly in the sections, the thin cartilage forming the roof of the myodomic groove runs gradually into membrane (fig. 17), the entire basis cranii thus here being perforated by a longitudinal opening that might be considered to be a fenestra ventralis myodomus. This is, however, not the case, for the recti externi lie definitely in this opening and not above it. The bounding walls of the opening accordingly represent the side walls of the myodomic groove, and the space between the ventral edges of the side walls alone represents the fenestra ventralis myodomus. The space between the dorsal edges of the opening is a perforation of the floor of the primordial cranium, and the membrane extending horizontally between the edges forms part of the floor of the cavum cerebrale cranii and also the roof of the dorsal compartment of this basioccipital portion of the myodome. The recti externi lie between this membrane and the parasphenoid, and they are still separated from each other by a median vertical membrane. The saccus vasculosus is large, lies in the cavum cerebrale cranii, and projects posteriorly slightly beyond this point.

Proceeding anteriorly in the sections to the region between the saccus vasculosus and the hypophysis (fig. 16), the membranous roof of the myodome becomes somewhat arched, and it now has its attachment, on either side, on the dorso-internal surface of the cartilage of the basis cranii, at some distance dorsolateral to the midventral perforation of the cartilage, that perforation now being definitely a fenestra ventralis myodomus.

A second membrane, evident also in the preceding figure, here closes the fenestra ventralis myodomus, the parasphenoid lying directly against its ventral surface. The median vertical membrane still extends between these two membranes, separating the myodome into lateral halves.

Still farther forward in the sections, in the region of the hind end of the hypophysis (fig. 15), the median portion of the membrane that forms the roof of the myodome, and hence also the median portion of the floor of the cavum cerebrale cranii, gradually descends, between the recti externi, on to the membrane forming the floor of the myodome, the two membranes fusing with each other there, and so forming a thick membrane which is both the median portion of the floor of the cavum cerebrale cranii and the median portion of the membrane closing the fenestra ventralis myodomus, the parasphenoid lying directly upon its ventral surface. The myodomic cavity, which here still belongs only to the dorsal compartment of the myodome, is thus separated into lateral halves, the hypophysis projecting ventrally between the two halves of the compartment, and each half lodging the related musculus rectus externus.

Still farther forward in the sections (figs. 13 and 14), the hind ends of the recti interni appear between the parasphenoid and the membrane closing the fenestra ventralis myodomus, that membrane thus being the horizontal myodomic membrane, and the space beneath it the ventral compartment of the myodome. The membrane forming, on either side, the roof of the related lateral half of the dorsal compartment of the myodome, now has its mesial attachment on the dorsal surface of the horizontal myodomic membrane, the latter membrane thus, in its lateral portions, separating the two compartments of the myodome, while its median portion forms part of the roof of the ventral compartment of the myodome and part of the floor of the cavum cerebrale cranii. The conditions here are accordingly similar to those in the 63-mm. specimen of *Trigla hirundo*.

Still farther forward, that lateral part of the horizontal membrane that, on either side, separates the two compartments

of the myodome, breaks down, but its median portion still persists as part of the floor of the cavum cerebrale cranii, and, anterior to the hypophysis, it is perforated by the internal carotids in their passage from the myodome into the cavum cerebrale cranii.

Thus the prootic bridge of this small specimen of *Cottus scorpius* is nowhere formed by cartilage, and if it be of cartilage in the adult, it must be a later chondrification of the membrane that, in this specimen, forms the floor of the cavum cerebrale cranii. That this does take place is probable, for a cartilaginous prootic bridge is developed relatively late in other fishes also, as will be explained later.

In a 37-mm. specimen of *Clinocottus analis* the conditions resemble those in *Cottus scorpius*, differing only in that the horizontal myodomic membrane takes no direct part in the formation of the floor of the cavum cerebrale cranii, simply arching upward to such an extent that it is in contact with, and partly fused with, the membranous prootic bridge, thus separating the myodome into median and lateral, instead of dorsal and ventral compartments (fig. 19).

The internal carotid arteries of *Cottus* and *Clinocottus* are strictly similar in their course and branches to those of *Trigla*. The cross-commissure of the efferent pseudobranchial arteries has a position strictly similar to that of the latter fish, and the nervus palatinus facialis of *Cottus* is as in *Scorpaena*, while that of *Clinocottus* is as in *Trigla*. An anterior portion of the ascending process of the parasphenoid has the position of, and replaces, the alisphenoid of *Amia*.

In none of these small specimens of the Loricati is either the dorsal or the ventral compartment of the myodome definitely closed toward the orbit by membrane, as, in my earlier work on these fishes, I said was the case in the adults. Both in embryos and the adult the spinal portion of each compartment opens into the prespinal portion, and in embryos this latter portion is largely open toward the orbit. In the adult the myodome is doubtless closed toward the orbits by connective tissues which develop around the rectus muscles as they enter it. In the

adults of these fishes I described a well-developed trigemino-facialis recess. In embryos this recess is not evident, but it must necessarily exist, potentially.

SYNGNATHUS ACUS

In a 115-mm. specimen of this fish the myodome and the fenestra ventralis myodomus are both limited to the prootic region. Posterior to the hind end of this fenestra is a shallow median groove on the ventral surface of the cartilaginous basis cranii, which extends into the basioccipital region and there lodges the hind end of the parasphenoid. This bone is triangular there, in transverse section, the apex of the triangle directed dorsally. In sections passing through the posterior portion of the fenestra ventralis myodomus, the parasphenoid is still triangular, and the cartilage bounding the fenestra on either side becomes entirely enclosed in perichondrial bone which forms part of the prootic. The parasphenoid lies directly between the ventromesial edges of these prootic bones, dense connective tissue filling the space between the parasphenoid and either prootic and also extending dorsally across the parasphenoid, there filling and closing the fenestra ventralis myodomus. Proceeding anteriorly in the sections, the parasphenoid becomes flatter and wider, and the cartilage in the ventral ends of the prootics vanishes. Further forward in the sections, a little space appears in the dense connective tissue that covers the dorsal surface of the parasphenoid, and in this space the hind ends of the recti externi soon appear (fig. 23), lying directly above the parasphenoid and separated from the cavum cerebrale cranii by membrane which continues the full length of the myodome and represents the prootic bridge.

Proceeding forward from this point, the parasphenoid begins to widen and at the same time to thicken dorsoventrally, and it soon has, in sections, a median circular portion with laterally projecting flanges, each flange being formed of external and internal plates which receive the ventral end of the ventral process of the prootic between them (fig. 22). In the rounded median portion of the bone a median cavity forms, and lodges

the hind ends of the recti interni, the recti superiores arising from the lateral walls of this cavity, near its anterior end. The recti externi lie dorsal to the parasphenoid, between it and the membrane which everywhere forms the roof of the myodome.

Still further forward, the bony roof of the median cavity in the parasphenoid is gradually replaced by a horizontal myodomic membrane which separates the myodome into two compartments, a dorsal one lodging the recti externi and a ventral one lodging the recti interni and superiores, the ventral compartment forming a semicircular depression in the floor of the entire myodomic cavity (fig. 21). The hind end of the ventral compartment is thus completely enclosed in the parasphenoid, and it seems absolutely certain that that part of the bone forming the roof of this compartment is simply an ossification of the horizontal myodomic membrane.

Proceeding anteriorly, the region of the ascending processes of the parasphenoid is soon reached, these processes rising to the level of the membranous roof of the myodome and suturing with the ventromesial edges of the prootics (fig. 20). The myodome is here semicircular in transverse section, its side wall and floor being formed wholly by the parasphenoid and its roof by membrane that separates it from the cavum cerebrale cranii. A median vertical membrane here descends from the membranous roof of the myodome, and in connection with it the recti inferiores have their origins.

The course and the main branches of the internal carotid artery are as in *Scomber*, except that the artery separates into its anterior and posterior divisions while still within the canal in the median vertical myodomic membrane, and that the anterior division then immediately enters the tissues forming the floor of the cavum cerebrale cranii, thus not actually entering the latter cavity.

HIPPOCAMPUS GUTTULATUS

In a 20-mm. specimen of this fish the myodome begins posteriorly beneath a part of the basis cranii that is of cartilage lined, on either side, with perichondrial bone. The parasphenoid lies at a certain distance ventral to this part of the basis

cranii, the space between the two being filled with dense connective tissue which is bounded laterally at its dorsal edges by little projecting flanges of perichondrial bone developed in relation to the cartilage of the basis cranii. The hind end of the myodome lies in this tissue, and lodges the hind ends of the recti externi. Proceeding anteriorly from this point, the cartilaginous roof of the myodome soon vanishes and is replaced by a thick layer of fibrous tissue. Cartilage is, however, now found in the ventral portion of each lateral wall of the myodomic cavity, this cartilage being enclosed between projecting flanges of the parasphenoid, one of these flanges lying along the external surface of the cartilage and the other along its internal surface. The internal flange lies in the fibrous tissue that lines the myodome, and is certainly developed in relation to it.

The internal carotid artery traverses its foramen at the hind edge of the ascending process of the parasphenoid, and then immediately enters and runs upward in the median vertical myodomic membrane, its course and distribution there being as in *Sygnathus*. The recti interni, superiores and inferiores have their origins anterior to this ascending column of the artery, close together, from the dorsal surface of the parasphenoid. The ventral compartment of the myodome is thus here wholly prespinal in position, for the foramen for the internal carotid artery lies in the plane of the pituitary opening of the brain case. The relations of these two openings to each other varies considerably in different fishes, the foramen for the artery lying markedly anterior to the pituitary opening in *Scorpaena*, but posterior to that opening in *Scomber*.

CATOSTOMUS

In *Catostomus teres*, Sagemehl ('91) describes a myodome that is everywhere closed ventrally by the parasphenoid, is bounded dorsally by the horizontal processes of the prootics, and apparently extends posteriorly slightly into the basioccipital. The basioccipital has a large pharyngeal process, perforated by a short canal which encloses the dorsal aorta, and Sagemehl

says ('91, p. 516) that this relation to the aorta at once suggests a lower vertebral arch. He, however, says that he finds weighty reasons against the assumption that it is such an arch. One of these reasons is that, excepting in this region of the Cyprinidae and in the tail region of all fishes, the lower arches always enclose the body cavity, and not simply the aorta. A second reason is that he himself finds, in embryos of *Chondrostomus nasus*, the pharyngeal process not preformed in cartilage, as the lower arches always are. Sagemehl accordingly concludes that the pharyngeal process of the Cyprinidae is not a lower vertebral arch, and he considers it to be a bone formed by the fusion of pharyngeal bones of dermal origin with another bone formed by the ossification of a ligament which, in the Characinidae, extends from the hind end of the basis cranii to the swim-bladder, embracing the aorta in its course.

In a 57-mm. specimen of *Catostomus occidentalis* I find the pharyngeal process formed by two ventrally projecting longitudinal flanges of bone which arise from a layer of bone surrounding the notochord, and, diverging slightly and straddling the aorta, abut against and fuse with the dorsal surface of a curved and porous plate which lies parallel to the dorsal surface of the pharynx (fig. 29). The aorta is thus enclosed in a canal that corresponds strictly to the aortal groove of *Hyodon*, except in that it is closed ventrally by the formation of a horizontal floor across its outer edges, and if the one is of vertebral origin, as I consider it to be, the other certainly also is. Whether the floor of the canal has been developed in primary continuity with its lateral walls, or as an independent dermal formation, as Sagemehl suggests, cannot be told from my sections. The lateral walls of the canal are prolonged anteriorly beyond its floor, and the aorta there lies (fig. 28) in an open groove similar to that of *Hyodon*, the lateral walls of the groove gradually diminishing in height and vanishing approximately in the level of the anterior end of the persisting notochord. Anterior to the point where the vacuolated contents of the notochord can last be recognized in the sections, the notochordal space still continues a certain distance, and in sections

passing through this region the hind ends of the parasphenoid are cut, one lying on either side of a median ridge on the ventral surface of the basioccipital, the median dorsal aorta lying ventral to the ridge (fig. 27). Here, unfortunately, one or more sections are missing in my series. In the next anterior existing section (fig. 26) there is a circular space in the basioccipital, in exactly the position of the notochordal space in the next posterior section of the series, but somewhat larger than it, and this space lodges the hind ends of the recti externi. The aorta here begins to separate into a lateral dorsal aorta on either side.

Proceeding anteriorly in the sections, the myodomic cavity increases in size, and that part of the basioccipital in which it lies forms a large median, dorsally projecting, and rounded ridge. The parasphenoid now extends across the median line (fig. 25). Still farther forward the fenestra ventralis myodomi begins, the parasphenoid closing it ventrally and having a broad but low median ridge which projects upward into the fenestra. This median ridge then sends upward a longitudinal ridge on either side, and in the space between these two ridges the recti interni make their appearance, separated from the recti externi by loose connective tissue which does not form a definite membrane (fig. 24).

Farther forward, the two lateral ridges on the dorsal surface of the parasphenoid vanish, leaving a flat median ridge, and the hind end of the hypophysis is there cut in the sections. This latter organ is large, lies wholly in the myodome, and projects posteriorly ventral to the roof of the myodome, here formed by the horizontal processes of the prootics. Anterior to the anterior edges of these latter processes the hypophysis is connected with the brain by a small stalk of nervous material, which perforates the membrane which there forms the roof of the myodome and the floor of the *cavum cerebrale cranii*.

The myodome of this fish is thus, up to this point, strictly normal, except that the hind end of its dorsal compartment is enclosed in the basioccipital, and that the recti externi apparently have their origins on the anterior end of the notochord instead of ventral to it.

In sections immediately anterior to those that cut through the nervous stalk of the hypophysis, the median ridge on the dorsal surface of the parasphenoid extends upward to the membranous floor of the cavum cerebrale cranii, and so occupies the position of a basisphenoid, which bone is said by Sagemehl to be absent in all of the Cyprindidae. A slight line separates this projecting process from the remainder of the parasphenoid, vaguely suggesting a fusion of two bones.

BLENNIUS GATTORUGINI

In *Blennius gattorugini* I described ('09) a myodome, the roof of which was said to be formed by membrane. This is correct, but it was also said that this membrane was attached, on either side, to the dorsal edge of a groove on the ventral edge of the prootic, and that that edge which represented the horizontal process of the bone. This is incorrect, for, on reexamining my material, I find that this groove simply lodges the related portion of the lateral edge of the parasphenoid and that the membrane representing the horizontal processes of the prootics is attached to a slight ridge on the side wall of the cranial cavity at a somewhat higher level, in direct posterior continuation of the line of the horizontal portion of the basisphenoid. The cavity thus formed lodges the recti externi only, and hence represents the dorsal compartment of the myodome. Having no sections of this fish, the arteries, veins and nerves, and the myodomic membranes could not be properly traced, but the conditions are apparently similar to those in *Hippocampus*, above described. Starks ('05) says that in six genera of the *Bleniidae* examined by him there was no myodome.

ARGYROPELACUS

In *Argyropelacus* a myodome is frequently referred to by Handrick ('01), but not particularly described, and I made brief reference to it in my work on the mail-cheeked fishes. The neurocranium of this fish is said by Handrick to be wholly of cartilage, no bone being found in any part of it, and the myodome lies external to this chondrocranium. Its hind wall is

said to be formed by the anterior, external wall of the bulla acustica, and it lodges the extracranial semilunar and ciliaris ganglia. The posterior portion of its roof is evidently formed by a horizontal plate of cartilage shown, in the figures given by him (*l. c.*, figs. 7 to 9, Pl. 1), lying ventral to the hypophysis, and which must accordingly represent the prootic bridge. In a figure of a transverse section in the postfacialis region a ventrally projecting process is shown at each lateral edge of this prootic bridge, and its ventral portion has apparently been cut off in the figure. These two processes certainly represent transverse sections of ventral processes of the prootics, similar to those found in other Teleostei, and they must form the lateral walls of the so-called extracranial myodome. Each process lies mesial to the foramen faciale of its side, and lateral to this foramen and also lateral to the foramen trigeminum there is a slight ridge of cartilage which must represent a dorsal portion of the lateral wall of the pars jugularis of a trigemino-facialis chamber. The ganglion trigeminum thus probably lies in the orbital opening of this chamber and not in the dorso-lateral corner of the myodome, as Handrick concluded. The basis cranii is perforated by a so-called 'Pituitargrube,' which is said to extend from the foramen trochleare nearly to the foramen trigeminum, is shown closed by a membrane which is perforated by the nervi optici, and extends posteriorly to the anterior edge of the prootic bridge. This so-called pituitary fossa is thus simply a perforation of the primitive cranial wall which has been formed by the fusion of the pituitary opening of the brain case with the foramina optici.

Supino ('01), in a work I did not have at my disposal when my paper on the mail-cheeked fishes was sent to press, finds several bones developed in relation to the neurocranium of this fish, two of them being the prootics and one the parasphenoid. This latter bone must evidently lie ventral to the myodome, and in a figure giving a ventral view of the entire neurocranium, extensive ventral processes of the prootics are shown which must form the lateral walls of the myodome. The foramina for the nervi trigeminus and facialis are said to perforate the

prootic, but they are not shown in the figure. It is however probable that the conditions resemble those in *Hyodon*, the myodome evidently being large and having a large orbital opening on either side. Whether or not there are both dorsal and ventral compartments to the myodome cannot be told, but they are probably both present. Supino says that a basisphenoid is found, which must accordingly separate the so-called pituitary fossa of Handrick's descriptions into a pituitary opening of the brain case and a foramen formed by the fusion of the foramina optici, and he adds that: "Posteriormente l'estremità delle porzione impari del basisfenoide si congiunge, nel *Chauloides* e *Argyropelacus*, con la cartilagine che si trova nella cavità dei muscoli oculari." This, while not quite clear, would seem to mean that cartilage formed some part of the floor of the myodome.

ESOX

In the adult *Esox* the myodome is large and extends posteriorly into a conical excavation in the anterior end of the basioccipital, as Huxley ('71) has stated. A horizontal membrane separates it into dorsal and ventral compartments, the dorsal one being large and lodging the recti externi, while the ventral one is short, extending, posteriorly only to the hind edges of the foramina for the internal carotid arteries. From there the membrane separating the two compartments rises rapidly to the ventral surface of the relatively deep membranous pituitary sac, fuses with that surface, and then appears as two separate membranes, each having its mesial attachment on the ventrolateral surface of the sac. The recti interni have their origins beneath this membrane, on the floor of the ventral compartment of the myodome, near its hind end. The recti superiores have their origins from the anterior edge of each half of the horizontal membrane, near its dorsomesial end, and the recti inferiores from the lateral walls of the spreading dorsal end of the median vertical membrane, immediately anterior to the membranous pituitary sac. This median vertical membrane is partly fused with the median portion of the hori-

zontal membrane, and it is traversed by the internal carotid arteries. The membranous roof of the subpituitary portion of the myodome extends forward from the horizontal processes of the prootics to the hind edge of the horizontal plate of the basisphenoid. The conditions in this fish are thus wholly normal.

Starks ('05) says that the dorsal end of the basisphenoid (dichost, Starks) of this fish is 'free,' and he suggests that this should be examined in connection with a 'myodome septum', formed of connective tissues, said by him to be found in this region and to be continued forward as the interorbital septum. Just what this myodome septum is is not clear, but it would seem to be the membranous roof of the myodome. Starks further says that "the dichost (=basisphenoid of Huxley) is always absent when the myodome is." No particular cases are cited, but it is evidently assumed that there is no myodome whenever a prootic bridge (shelf) is not found in the prepared skeleton of the cranium. This is incorrect, and the statement should probably be that there is no basisphenoid whenever the roof of the myodome is wholly of membrane. Whether or not this statement is true, even in this form, I do not know, my material being too limited to permit me to form an opinion.

GASTEROSTEUS ACULEATUS

The early stages of the development of the myodome in *Gasterosteus aculeatus* are quite fully described, and the myodome of the adult briefly described by Swinnerton ('02). The trabecula and parachordal of either side are said by him to be, when first formed, wholly independent cartilages, and their adjoining ends are shown in the figures lying approximatively in the tranverse plane of the tip of the notochord. The posterior halves of the trabeculae are said to enclose the infundibulum and the pituitary body, and the infundibulum is shown lying posterior to the pituitary body and reaching to the tip of the notochord. The trabeculae and parachordals soon fuse with each other, and there is then a marked anterior growth of the parachordals which carries the trabeculae forward considerably

anterior to the tip of the notochord. The pituitary body still lies between the hind ends of the trabeculae, in the so-called pituitary fossa, but the infundibulum now lies dorsal to the anterior end of the notochord. The space between the anterior ends of the parachordals is now called the interparachordal fossa, and this and the pituitary fossa are not only continuous with each other in these early stages of development, but are considered to continue so to be even in the adult. As the term fossa is probably here employed strictly in the sense of fenestra, these two so-called fossae will hereafter be referred to as the fenestrae interparachordalis and hypophyseos.

In the third and fourth stages considered by Swinnerton (embryos 6.6 to 25-mm. in length) it is said that the intracranial notochord has undergone no further change beyond a slight increase in absolute length, and further, that it undergoes no actual suppression or reduction even in later stages of development. It is also said that: "The interparachordal fossa has been carried some distance in front of the notochord; and the parachordals themselves have united across the intervening space and across the end of the notochord in such a way that this projects below, but close against the basis cranii." A transverse plate of parachordal cartilage is thus formed, and a median sagittal section through it in a 14-mm. specimen is shown in one of the figures given (*l. c.*, fig. 38, pl. 30.). The parasphenoid is shown lying slightly below the parachordal plate, and the hind end of the musculus rectus externus is inserted on the dorsal surface of the parasphenoid beneath the anterior edge of the plate. Somewhat anterior to this point, a process of bone is shown projecting dorso-anteriorly from the dorsal surface of the parasphenoid, and it is called the median process of that bone. In the space between this process and the anterior edge of the plate of parachordal cartilage, a section of the basal portion of the brain is shown, and, although not index lettered, it must represent the pituitary body and infundibulum of the earlier stages, the infundibulum here slightly differentiated as the saccus vasculosus. In a sagittal section through this same region of the adult (*l. c.*, fig. 37), the rectus

externus is shown lying between the parasphenoid and the plate of parachordal cartilage, and extending posteriorly beyond the prootic region into the anterior end of the basioccipital region. The plate of parachordal cartilage now forms a prootic bridge, but how it has been developed is not explained. In the base of the median process of the parasphenoid is a block of cartilage said to represent the anterior end of the parachordal, the median process of the parasphenoid thus lying posterior to the fenestra hypophyseos. The pituitary body (hypophysis) and infundibulum (saccus vasculosus, both shown lying posterior to the median process of the parasphenoid, must then also lie posterior to the fenestra hypophyseos, and this is apparently also their position in advanced embryos and the adults of certain other, if not all fishes, as will appear later.

Swinnerton does not describe the internal carotid arteries, but it seems certain, both from his figures and from the conditions in a 40-mm. specimen of this fish, described immediately below, that these arteries pass upward between the hind ends of the trabeculae and that they are never there enclosed in cartilage.

In the earlier stages considered by Swinnerton the rectus externus muscles are said to be inserted into each other and into the tissues filling the hind part of the fenestra interparachordalis. In the third and fourth stages they extend posteriorly so that their hind ends lie beneath the posterior border of that fenestra, and hence along the ventral surface of the basis cranii. The eyeballs have in the mean time descended to a level relatively lower than in the earlier stages, and this is said to cause the eye-muscles to press upon the anterior prolongations of the parachordals and, depressing them, institute the beginning of the formation of the myodome. It would naturally be supposed that this depression would affect the hind ends of the trabeculae, with which the parachordals are fused, and this is what actually takes place in *Salmo*, as described by Gaupp and to be later considered. In *Gasterosteus*, on the contrary, the hind ends of the trabeculae have not been in the least depressed in the oldest stages shown by Swinnerton in which

they still persist, an embryo belonging to his third stage and said to be 6.6 mm. in length (*l. c.*, fig. 8). The lateral edges of the anterior prolongations of the parachordals are also not affected, as shown in that figure, their mesial edges alone being depressed. This depression of the edges must then represent a ventral growth of the cartilage, for it is difficult to comprehend how it could have been the result of any pressure of the rectus muscles.

In Swinnerton's fourth stage, those parts of the trabeculae which border on the fenestra hypophyseos are said to have been suppressed, and it is said (*l. c.*, p. 518) that:

In the hinder or parachordal portion, the interparachordal fossa has been carried so far away in front of the notochord that the plate formed by the median union of the parachordals now furnishes a considerable portion of the basis cranii. Those parts lying immediately on either side of the fossa have now begun to undergo a movement of depression, by which they have already come to lie slightly below the level of the basis cranii.

Swinnerton says that this movement of depression is perhaps associated with a similar movement on the part of the rectus muscles, but, as just above stated, this ventral growth of the parachordal cartilage begins in earlier stages, and it seems improbable that it can there be due to any action of these muscles.

In later stages of development, it is said (*l. c.*, p. 527) that:

The process of depression of those parts bounding the interparachordal fossa laterally has continued, so that this region now appears to be a mere downward process of the prootic, with its cartilaginous extremity mortised into the sides of the parasphenoid. This appearance is enhanced by the fact that posteriorly each process is continued into a ridge running along the under surface of the hinder portion of the prootic. These two ridges are continuous with those already described under the basioccipital, and there is a channel thus formed which runs a considerable length of the basis cranii, is closed ventrally by the parasphenoid, and opens anteriorly into the cavum cranii by means of the interparachordal fossa.

It is then further said (*l. c.*, p. 528) that:

In the larval *Amia* this canal is not present, but there is a well-marked interparachordal fossa to which the eye muscles bear the same

relations as in the stickleback. It is probable, therefore, that in this fish also a process of depression and secondary growth goes on on either side of the fossa and below the prootic; but that, whereas in the other types the fossa persists and transmits the eye muscles back again out of the cranial cavity beneath the basis cranii, in *Amia* it disappears, owing to continuous cartilaginous growth. As far back as the so-called prootic bridge these muscles may be said to run in an actual derivative of the cranial cavity; behind that they run in an extracranial space secondarily enclosed.

The myodome of *Gasterosteus* is thus conceived by Swinnerton to be a space, the anterior portion of which is bounded laterally by the bent-down anterior prolongations of the parachordals, and the posterior portion by secondary ventral downgrowths of the parachordals posterior to those anterior prolongations. These two portions of the myodome are thus of totally different origin, and the anterior portion is considered, because of its relations to the parachordals, to be an actual derivative of the *cavum cerebrale cranii*. The figures given show that it lodges the pituitary body, and that it is prechordal in position, but, as the arteries and veins of the region are not shown or particularly described, it is impossible to compare the conditions here with those in the fishes that I have considered above. I have accordingly examined this region in a series of transverse sections of a 40-mm. specimen of this fish, and as the conditions there present certain new features, they will be quite fully described.

In this 40-mm. specimen of *Gasterosteus* I find the recti superiores, inferiores, and interni all arising from a thick median vertical membrane which descends from the ventral surface of the anterior portion of an unusually large membranous pituitary sac, the recti interni having their origins posteroventral to the recti superiores and inferiores. Posterior to the points of origin of these muscles, the large hypophysis projects ventrally into the membranous pituitary sac, which lies dorsal to, and in large part posterior to, the dorsoanterior edge of that transverse ridge on the dorsal surface of the parasphenoid which Swinnerton calls its median process. This process begins near the hind edges of the ascending processes of the parasphenoid,

and, projecting dorso-anteriorly, extends approximately to the transverse plane of the anterior edge of the hypophysis, where it reaches to about the middle of the height of the myodome. The large membranous pituitary sac rests upon its dorsal surface, that surface being presented dorsoposteriorly. The space beneath this ridge opens anteriorly into the subpituitary portion of the myodome.

The ascending processes of the parasphenoid have their greatest dorsal extent anterior to the transverse ridge on its dorsal surface, and Swinnerton says that these processes of *Gasterosteus* are not the homologues of the processes of the bone of *Amia*. Swinnerton based this conclusion wholly upon the fact that each process of the bone of *Gasterosteus* lies anterior to the foramen for the nervus trigeminus, while in *Amia* it lies posterior to it; but he overlooked the fact that a part of the process of *Gasterosteus*, as shown in his figure 19, plate 29, projects dorsally posterior to the foramen trigeminum, this part of the process thus corresponding to the process of *Amia*. The anterior portion of the process of *Gasterosteus* lies lateral to the oculomotorius, trochlearis, and profundus nerves, and also lateral to the vena jugularis and the rectus muscles, between them and the nervus trigeminus, thus having exactly the relations to these several structures as does the pedicel of the alisphenoid bone of *Amia*. This part of the process of *Gasterosteus* thus replaces functionally a pedicel of the alisphenoid, and it has certainly been developed in relation to tissues that represent, in this fish, that bone of *Amia*. The orbital opening of the myodome of *Gasterosteus* thus differs from the opening in all the other fishes so far considered, except *Cottus* and *Clinocottus*, in which latter fishes the pedicel of the alisphenoid of *Amia* is also represented by a process of the parasphenoid. The orbital opening of the myodome of *Gasterosteus*, and also that of *Cottus* and *Clinocottus*, does not, however, correspond strictly to that of the myodome of *Amia*, for, as will be shown later, there has been added to its ventral portion the canals traversed, in *Amia*, by the internal carotid arteries and the palatine branches of the faciales.

In my 40-mm. specimen of *Gasterosteus* the internal carotid and efferent pseudobranchial arteries of either side perforate the base of the ascending process of the parasphenoid through a single foramen, and enter the space beneath the transverse ridge on the parasphenoid. There the pseudobranchial artery is connected by a cross-commissure with its fellow of the opposite side, and then runs forward into the orbit as the *arteria ophthalmica magna*. The internal carotid gives off, before entering its foramen, its orbitonasal branch, which traverses the foramen with the internal carotid and efferent pseudobranchial arteries, and then runs forward along the floor of the myodome to enter the orbit. The internal carotid, after giving off this branch and having entered the space beneath the transverse ridge on the parasphenoid, turns upward in the median vertical myodomic membrane, and, while in that membrane, anastomoses with its fellow of the opposite side. It then separates from its fellow and, while still in the membrane, divides into two parts, one of which at once enters the *cavum cerebrale cranii*, and is the posterior cerebral artery. The other part runs forward in the thick median portion of the membranous floor of the *cavum cerebrale cranii*, and, issuing beneath it, sends two branches to the eyeball, one of them accompanying the *nervus opticus*. The remainder of the artery then enters the *cavum cerebrale cranii* through the foramen opticum, and is the anterior cerebral artery. No positive and definite connection between the anterior and posterior cerebral arteries was seen, the anterior branch of the latter artery, found in the other fishes, not occurring here.

The *ramus palatinus facialis* arises from the trigemino-facialis ganglionic complex, and passing lateral and then ventral to the *vena jugularis*, runs ventromesially along the internal surface of the prootic bone and perforates the dorso-anterior portion of the transverse ridge on the parasphenoid to enter the space beneath it and then to escape into the orbit. This nerve, in this fish, thus lies lateral to the *vena jugularis*, while in all others in which it was traced (*Hyodon*, *Scomber*, *Scorpaena*, *Cottus*, *Catostomus*, and *Amia*) it lies mesial to that vein. This is, how-

ever, unquestionably related to the fact that the vena jugularis lies ventral (mesial) to the nervus facialis in *Gasterosteus*, while in the other fishes mentioned above, except *Catostomus*, it lies dorsal (lateral) to that nerve. In *Catostomus* the vein lies ventral (mesial) to the nervus facialis but lateral to the nervus palatinus, this thus being a variation in the transformation of the primitive vena cardinalis anterior into a vena capitis lateralis.

A delicate median vertical membrane separates the space beneath the transverse ridge on the dorsal surface of the parasphenoid into lateral halves, this membrane being continuous anteriorly with the membrane that gives insertion to the rectus muscles. At its hind end this membrane ossifies as a short median ridge on the dorsal surface of the parasphenoid.

The space beneath the dorso-anteriorly projecting ridge on the dorsal surface of the parasphenoid thus corresponds strictly to the ventral compartment of the myodome of the other Teleostei so far considered, but the roof of that compartment, which is of membrane in those other fishes, has here been ossified as part of the parasphenoid. The recti externi and the pituitary veins run posteriorly dorsal to this ridge, and hence lie in the dorsal compartment of the myodome, the pituitary veins lying along the lateral surfaces of the pituitary sac, and forming, posterior to it, a large median sinus.

Posterior to the hind end of the ventral compartment of the myodome, and hence posterior also to the ascending processes of the parasphenoid, a tall median ridge of the latter bone, flat on its dorsal surface, projects upward between the ventral ends of the ventral processes of the prootics, its dorsal surface there forming the floor of the dorsal compartment of the myodome. Up to this point the ventral processes of the prootics are wholly of bone, but cartilage now appears in their ventral halves, as shown in Swinnerton's figure 35, plate 30. The hind end of the membranous pituitary sac is here cut in the sections. Proceeding posteriorly, the median ridge on the dorsal surface of the parasphenoid gradually becomes wider, and, arching upward, projects into the myodome between the ventral ends of the ventral processes of the prootics. The membrane which,

anterior to this point, formed the roof of the myodome, is now replaced by membrane bone which forms the anterior portion of the prootic bridge, and the nervus abducens perforates it, on either side, to enter the dorsal compartment of the myodome. Farther posterior in the sections, a median plate of cartilage appears in the prootic bridge, enclosed between dorsal and ventral plates of perichondrial bone, and in this transverse plane the cartilage in the ventral ends of the ventral processes of the prootics disappears and is replaced by membrane only. The ventral processes of the prootics are accordingly now formed by short processes of bone, partly of membrane and partly of perichondrial origin, that are prolonged ventrally by membranes, continuous ventrally with the lateral edges of the parasphenoid. Proceeding posteriorly, the median plate of cartilage expands laterally, on either side, and becomes the cartilaginous basis cranii, here still enclosed, on either side, between plates of perichondrial bone which form parts of the prootics. The myodome still continues onward, in a median groove on the ventral surface of this cartilage, there lodging the recti externi and being bounded laterally in part by membrane only and ventrally by the parasphenoid.

At the extreme hind end of the myodome a circle of bone appears in the sections, this bone forming part of the basioccipital and lying in the groove on the ventral surface of the cartilaginous basis cranii. From this shell of bone a median plate is sent downward between the diverging hind ends of the parasphenoid, and the shell of bone then fuses with perichondrial bone developed in relation to the overlying cartilage and forming part of the basioccipital. The conditions are thus here practically as described and figured by Swinnerton in his 16-mm. specimen of this fish (*l. c.*, fig. 36, pl. 30).

The myodome of *Gasterosteus* is thus strictly comparable to that in the other Teleostei described, except that it has a greater anterior extension than it has in any of them, *Cottus* and *Clinocottus* excepted, this being due to the ossification, as part of each ascending process of the parasphenoid, of tissues representing the pedicel of the alisphenoid. The parasphenoid has under-

gone special development in this fish, and the conditions here show, even more positively than in the others considered, that part of this bone may be developed in definite relations to the membrane separating the myodome into dorsal and ventral compartments. It is accordingly certain that this bone is here developed, in part, in relation to axial skeletogenous material, and is not a simple dermal bone primarily developed in relation to the mucous lining membrane of the pharynx, and which sank gradually inward to its actual position.

DACTYLOPTERUS VOLITANS

The conditions in *Gasterosteus*, as above explained, seeming to offer an explanation of the somewhat exceptional conditions that I described in *Dactylopterus* in my work on the mail-cheeked fishes, I have reexamined my material of that fish. In that earlier work I described a transverse ridge on the dorsal surface of the parasphenoid that was said to project dorsoposteriorly and to form the posterior wall of the myodome. Because of the position of this wall, I concluded that the post-pituitary portions of the horizontal processes of the prootics had been depressed and appressed upon the underlying ventral flanges of those bones, and that the latter flanges had undergone marked reduction. I now find that the ventral flanges of the prootics have not undergone any particular reduction, and that there has been no depression and appression of the horizontal processes of the prootics, which are represented by a well-defined membrane forming the floor of the *cavum cerebrale cranii*. The anterior end of this membrane passes over the dorsal edge of the transverse ridge on the parasphenoid, closely adherent to it, and is then continuous with the membrane that I described as closing the pituitary opening of the brain case. Beneath the part of this membrane that represents the horizontal processes of the prootics, and between it and the parasphenoid, is a space which must represent some part of the dorsal compartment of the myodome, this space being shut off from the subpituitary portion of the myodome by the transverse ridge on the parasphenoid. The hypophy-

sis lies anterior to this ridge, the cross-commissure of the pituitary veins lying ventral to the hypophysis and separated from it by the dura mater. The recti externi have their insertions on a median vertical membrane, immediately posterior to the pituitary veins and immediately anterior to the summit of the transverse ridge. They are surrounded by connective tissue that resembles the fatty tissue found abundantly in this fish, but are not otherwise separated from the other rectus muscles, the dorsal and ventral myodomic compartments thus apparently here being confluent.

SALMONIDAE

In *Salmo*, Parker ('73) and Stöhr ('82) found the trabeculae and parachordals primarily independent of each other. Stöhr also found the anterior portions of the parachordals—the parts corresponding to the anterior prolongations of the parachordals of Swinnerton's descriptions of *Gasterosteus*—primarily independent of the posterior portions, and he considered them to represent the 'Balkenplatten' of the Amphibia. They are said by him to fuse, first, with the posterior portions of the parachordals and then with the trabeculae. Of the adult *Salmo*, Parker says (*l. c.*, p. 102):

One remarkable change in the investing mass, as a whole, is the growth downward of a lamella on each side, thus forming a covered archway; for in front of the retiring notochord the moieties of cartilage meet, and this viaduct is floored by the submucous bone which has been removed, the parasphenoid. All the true axial parts of the skull cease at the front edge of the investing mass behind the pituitary space; all the rest has a facial foundation, is built on the trabeculae, or has a secondary character as a development of the cranial wall.

This so-called covered archway is the myodome, which is thus considered by Parker to be bounded laterally by downgrowths of the parachordal cartilage and not by those cartilages, themselves, bent down.

Schleip says ('04, pp. 355 to 359) that, in trout embryos, 12 to 14-mm. in length, the parachordals and trabeculae form the floor of the primordial cranium, and that the cartilages of

opposite sides are separated from each other by a fissure (Fissur) the posterior, interparachordal portion of which is entirely filled by the projecting anterior end of the notochord. There accordingly is, as described by Schleip, no interparachordal fenestra in these embryos. The intertrabecular portion of the fissure is called the pituitary fossa and it is said to be closed ventrally by the parasphenoid, which, at these stages, extends from the orbit only to the tip of the notochord (*l. c.*, p. 354). The rectus muscles, in running from their insertions on the bulbus to their points of origin, are said to lie, in their anterior portions, either above the trabeculae or above the fissure (so-called pituitary fossa), and farther posteriorly to lie in the fissure itself; the recti externi extending still farther posteriorly so that their hind ends lie under the notochord and hence beneath the basis cranii. That part of the space above the trabeculae, or above the fissure, thus occupied by the rectus muscles is said to form a part of the cranial cavity, but to be closed toward the brain by a membrane which, in these embryos, extends posteriorly to the tip of the notochord. Anteriorly, the edges of this transverse membrane are said to be attached to the side walls of the cranium, above its floor, the membrane thus there separating the cranial cavity into dorsal and ventral parts. Posteriorly, the membrane is said to stretch from one trabecula to the other, there closing the intertrabecular fissure, (the so-called pituitary fossa) and taking part in the formation of the basis cranii. It is, however, further said that, in later stages (embryos 18-mm. long), this same posterior portion of the membrane chondrifies, and that the cartilage so formed extends posteriorly to the tip of the notochord and there forms both the roof of the eye-muscle canal (myodome) and the floor of the cranial cavity. This cartilage is thus evidently the pro-otic bridge, and as the bridge cannot possibly have been formed by the chondrification of a membrane extending from one trabecula to the other, there is some error in the descriptions.

The myodome, as above described, is said by Schleip to present three sections: an anterior one, intracranial in position, but separated from the brain by the transverse membrane above

referred to; a middle section, "der in der Fissur der Schädelbasis, bezw. in einem nach unten offenen Sulcus liegt," and a posterior section which lies wholly beneath the basis cranii. Reference is here made by Schleip to a series of half schematic figures (*l. c.*, pp. 355 to 357), and consideration of them shows that the so-called anterior section of the myodome is what I have called, in the fishes described by me, its prespinal section. The middle section is apparently my prootic portion of the spinal section, and the posterior section what I have called the basioccipital portion of that section. A membrane is shown in these figures extending transversely between the ventral ends of the ventral processes of the prootic cartilages and separating the recti externi from the recti interni. This membrane is the horizontal myodomic membrane of my descriptions, but I cannot find that Schleip refers to it in his text, for the transverse membrane of his descriptions is said to form the roof of the myodome.

In a 25-mm. embryo of *Salmo salar* Gaupp finds conditions strikingly similar to those described by Swinnerton in *Gasterosteus*, and he arrives at practically similar conclusions regarding the development of the myodome, without, however, here making special reference either to that author's or to Schleip's conclusions regarding it. Like Schleip, Gaupp ('05 b, pp. 665 to 669) separates the myodome into anterior, middle, and posterior sections. The anterior section is said to lie in the posterior portion of the orbitotemporal region, and its floor to be formed by the two trabeculae and a membrane which extends transversely between them. The space between the two trabeculae is called by Gaupp the fenestra basicranialis anterior, or fenestra hypophyseos, and it corresponds to the intertrabecular, or pituitary fossa of Swinnerton's descriptions of *Gasterosteus*. It, however, apparently corresponds to the anterior portion only of the intertrabecular, or pituitary fossa of Schleip's description of the trout, the hind ends of the trabeculae of Schleip's account corresponding to the anterior prolongations of the parachordals of Swinnerton and Gaupp. The membrane said by Gaupp to close the fenestra hypophyseos of *Salmo* is shown by him, in a figure of a cross-section through this region

(*l. c.*, fig. 345, p. 669), lying ventral to the muscoli recti interni, and it is furthermore said that it gives attachment on its dorsal surface to the metachiasmatic (posterior) portion of the interorbital septum. The posterior portion of the interorbital septum here referred to is evidently the median vertical myodomic membrane of my account, the membrane that closes the fenestra hypophyseos then being represented in the layer of skeletogenous tissue forming the floor of what I have called the sub-pituitary portion of the ventral compartment of the myodome.

The roof of the anterior section of the myodome, as thus described by Gaupp, is said by him to be formed by the membranous floor of the cranial cavity, and its side walls by the ventral portions of the cartilaginous side walls of the cranium, which are said to here extend between the otic capsules and the trabeculae. The eye muscles are said to have forced the brain upward from the basis cranii, the hypophysis being carried with it and so lifted out of the fenestra hypophyseos.

The middle section of the myodome is said by Gaupp to lie, in part, in the labyrinth region and, in part, in the extreme posterior (hintersten) portion of the orbitotemporal region. Its floor is said to be formed by the anterior prolongations of the parachordals (vordere Parachordalia) which have been forced ventrally by the pressure of the muscoli recti externi, exactly as Swinnerton had previously said was the case in *Gasterosteus*. Gaupp, however, shows the hind ends of the trabeculae—the parts bounding the fenestra hypophyseos—forced ventrally to the same extent as the parachordals. Because of its relations to the anterior parachordals, this middle section of the myodome is said to lie between the primordial basis cranii and the brain, and hence to form a part of the primordial cranial cavity. Its side walls are described as formed, on either side, by two basicapsular commissures, which extend from the otic capsule of their side to the anterior prolongation of the related parachordal, and lie, one between the nervi trigeminus and facialis, and the other between the latter nerve and the otic capsule. Its roof is formed by the membranous floor of the cavum cerebrale cranii, this membrane arising, on either side,

from the side wall of the cranium, extending posteriorly dorsal to the notochord, and anteriorly passing into the supraseptal membranous floor of the *cavum cerebrale cranii* in the orbito-temporal region. In this membranous roof of this middle section of the myodome a transverse bridge of cartilage, the prootic bridge, is later developed, and it is said to lie above and anterior to the tip of the notochord. In the adult this bridge extends forward to the hind edge of the hypophysis, as shown in Parker's figure of a bisected skull ('73, fig. 4, pl. 7), and the pituitary opening of the brain case lies considerably posterior to the anterior edges of the ventral processes of the prootics. It must then be that, as in the adult *Gasterosteus*, the hypophysis of the adult *Salmo* lies dorsal to the interparachordal fenestra and not dorsal to the fenestra hypophyseos.

The middle section of Gaupp's descriptions of the myodome thus apparently corresponds to the subpituitary portion of the prespinal section, and to all of the prootic portion of the spinal section, of my descriptions. Gaupp says that, primarily, the *nervus palatinus facialis* issues from the cranial cavity along the lateral edge of the anterior parachordal, but that, as the myodome gains in height and breadth, the nerve becomes included in it, then entering it by perforating its membranous roof and leaving it through a foramen in its floor. The course of the internal carotid arteries is not given, but as there are no special perforations of the basis cranii for them, they must pass upward through the fenestra hypophyseos. Gaupp shows, in his figure of the entire chondrocranium, a foramen lying between the foramen for the *nervus facialis* and the incisura prootica, and it is said to give passage to the *vena jugularis*, coming from the anterior portion of the cranial cavity. This vein is, however, certainly not the *jugularis* of current descriptions of fishes, and is probably the encephalic vein of Allen's ('05) description of the *Loricati*. It cannot be the pituitary vein, for that vein does not extend into the anterior portion of the cranial cavity.

The posterior section of the myodome is said to lie beneath the basis cranii, between it and the parasphenoid, and to com-

municate with the middle section through the fenestra basicranialis posterior, which lies between the anterior ends of the parachordals and apparently corresponds to the posterior portion of the intertrabecular fissure of Scheip's descriptions. The recti externi pass through this fenestra, and, beyond it, lie beneath the basis cranii. There, as they increase in size, they are said to push both the middle portion of the basis cranii upward and the parasphenoid downward. The basal plate, formed by the parachordals and the enclosed notochord, then thickens along each lateral surface of these muscles, and so forms the lateral walls of this section of the myodome.

Comparing the conditions in this fish with those in *Gasterosteus*, it is seen that, in both fishes, the anterior portions of the parachordals lie, when first formed, at a certain distance lateral to the anterior end of the notochord, which projects anteriorly between them. In later stages of both fishes these projecting portions of the parachordals are said, by both Gaupp and Swinnerton, to be depressed, but the figures given by both show that this depression effects only the mesial edges of the parachordals, their lateral portions retaining their primitive positions in the level of the notochord. Between these higher lying portions of the cartilages, the prootic bridge is later developed. How this bridge is developed in *Gasterosteus* is not stated by Swinnerton. In *Salmo* Gaupp says it is formed by the chondrification of a part of the membrane forming the roof of the middle section of the myodome and also the floor of the cavum cerebrale cranii. Gaupp says it lies, when first formed, anterior to and above the tip of the notochord, and it is shown, in one of his figures, separated from that tip by the anterior portion of an open space that is prolonged posteriorly on either side of the anterior end of the notochord. The posterior portion of this space may possibly form part of the fenestra basicranialis posterior of Gaupp, but its anterior portion certainly does not, for Gaupp says that this fenestra lies between the edges of the bent-down parachordals and gives passage to the recti externi from the middle to the posterior section of the myodome. There are, then, four distinctly different fenestrae in this myodomic

region. One of them is the fenestra hypophyseos of Gaupp, which lies between the hind ends of the trabeculae and hence in the floor of the anterior section of the myodome; and this must be, in early embryos, traversed by the internal carotid arteries, for Parker ('73) shows those arteries, in this fish, running upward anterior to the hypophysis, and I so find them in all the Teleostei I have examined. A second fenestra is the fenestra basicranialis posterior of Gaupp, which lies partly in the floor of the middle section of the myodome and partly between that section and the posterior section. A third fenestra, not described by Gaupp, lies in the floor of the posterior section of the myodome, and this, together with that part of the second fenestra that lies in the floor of the middle section of the myodome, forms the fenestra ventralis myodomus, the so-called hypophysial fenestra of Sagemehl. The remainder of the second fenestra—the part leading from the middle section of the myodome into the posterior one—is simply a transverse section of the continuous cavity of the myodome and does not open on to the ventral surface of the cranium. The fourth fenestra lies in the roof of the middle section of the myodome, and this alone is the homologue of the fenestra basicranialis posterior of the Sauropsida. This is evident from Sonies's ('07) description of this fenestra in the chick and duck, to be discussed later, and from Gaupp's ('00) account of it in *Lacerta*. In *Lacerta* the fenestra is said by Gaupp to be bounded anteriorly by the crista sellaris, and to be closed by a membrane (Gewebe) everywhere continuous with the perichondrium of the bounding cartilages, and that represents an unchondrified portion of the primordial cranium. The anterior end of the notochord is enclosed in this membrane, and lies, in part of its course, so close to its ventral surface that it forms a longitudinal ridge along it. The fenestra accordingly lies in what corresponds to the roof of the myodome of fishes, and not to its floor, and hence cannot be the homologue of the similarly named fenestra of Gaupp's descriptions of *Salmo*. In the Urodela, also, the fenestra basicranialis posterior is said by Gaupp ('05 b, p. 692) to be a perforation of the basal plate, traversed by the notochord, and lies posterior to its tip, as it does in *Lacerta*.

AMIURUS

In the adult *Amiurus* the myodome was briefly considered by me in my work on the mail-cheeked fishes, and I there said (Allis, '09, p. 200) that;

In the anterior three-fifths, approximately, of its length, the ventral edge of the prootic does not meet its fellow of the opposite side, a wide hypophysial fenestra, closed ventrally by the parasphenoid, being left between the two bones. Posterior to this fenestra, the ventral edges of the prootics meet in the middle line, and the two bones there form, on the floor of the cranial cavity, a prominent transverse bolster which has closely the position of the cross-canal of *Lepidosteus*; and it is certainly in this bolster that McMurrich found the small cavity that he considered to be a rudimentary myodome.

In the specimens that I examined at that time I found but slight indication of this cavity, but I nevertheless considered it to have existed previously in the transverse bolster and to have been suppressed by invading growth of the surrounding cartilage.

In my work on the pseudobranchial and carotid arteries of this fish, I said (Allis, '08 b, p. 259) that the external carotid artery

does not apparently traverse a trigemino-facialis chamber, for although it would seem as if that chamber must be present in some form, there is no proper indication of but one cranial wall in this region, and that one wall would seem to be the inner wall of the chamber; for both the external carotid and the jugular vein lie external to it.

It was further said (p. 259) that:

The parasphenoid of *Ameiurus* is peculiar in that the base of the ascending process of the bone, which begins immediately posterior to the so-called orbitosphenoid, is formed of two plates which enclose within them the hind end of the subopticus (trabecular?) bar of cartilage. The bone is here apparently not of perichondrial origin, but the inner plate nevertheless lies internal to the cartilage of the skull and there forms part of the immediate bounding wall of the cranial cavity. Posterior to the hind end of the trabecular (?) cartilage there is, for a few sections, a vacant space between the two plates of the process of the parasphenoid, and then those plates, the inner one of which gradually diminishes in height, enclose the anterior portion of the prootic (parachordal?) cartilage. It is perhaps this portion of the bone of the adult that led McMurrich to conclude that the basisphenoid was here ankylosed with the parasphenoid.

I have now reexamined my sections of young specimens of this fish, but the material was evidently not in a good state of preservation when sectioned, for the membranes in the myodomic region are all more or less disintegrated. The cartilages I tentatively identified in my earlier work as trabecular and parachordal are certainly those cartilages, as currently conceived, for the one lies wholly anterior to the hypophysis and the other along the lateral wall of, and posterior to, that organ. The anterior end of the parachordal cartilage is, as I stated, enclosed between external and internal plates of the parasphenoid, but neither plate is adherent to it, and, in the adult, the cartilage lies in a little pocket on the dorsal surface of the parasphenoid and can be easily withdrawn from it without breakage. Posterior to this pocket, the cartilage, in embryos, gradually becomes enclosed between plates of perichondrial bone which form part of the prootic, the internal plate of the parasphenoid gradually diminishing in height and finally vanishing. The ventral edges of the prootics form the lateral boundaries of the fenestra ventralis myodomus. The hypophysis is large, lies directly above this fenestra, upon the dorsal surface of the parasphenoid, and extends posteriorly to the anterior surface of the transverse bolster described in my earlier work. This bolster is but slightly developed in my young specimens, but it is evidently formed either by the fusion of the horizontal and ventral processes of the prootics or by the horizontal processes alone, the ventral processes of the prootics, in the latter case, here vanishing. The cavity described by McMurrich ('84) in this bolster would then seem to represent the prootic portion of the dorsal compartment of a myodomic cavity. A ventral myodomic compartment is wholly wanting, for that part of the parasphenoid lying in the prootic region has certainly been developed in the skeletogenous tissue which, in the other Teleostei described, forms the horizontal myodomic membrane, this part of the parasphenoid of *Amiurus* thus corresponding to the transverse ridge on the dorsal surface of the bone of *Gasterosteus*. This, then, accounts for the fact that both the internal carotid artery and the ramus palatinus facialis of *Amiurus* lie everywhere external to the para-

sphenoid instead of passing internal to the ascending process of that bone. Whether or not there is a subpituitary portion of the dorsal myodomic cavity I cannot determine, the membranes being in a more or less disintegrated condition. It is, however, apparently wanting, for there are no veins comparable to the pituitary veins of the other Teleostei considered, the pituitary region being drained by veins definitely in the *cavum cerebrale cranii*. Furthermore, the membranous pituitary sac apparently forms the perichondrial lining of the pituitary fossa, as it also does of the larger part of that fossa in the *Selachii*. There is no *pars jugularis* of a *trigemino-facialis* chamber.

The internal carotid artery gives off, as described earlier (Allis, '08 b), an orbitonasal artery, sends two branches to the eyeball, and then enters the cranial cavity through the foramen opticum, behind the *nervus opticus*. This latter nerve certainly lies dorsal to the *trabecula*. The internal carotid artery of this fish must then also have that relation to that cartilage, the artery accordingly entering the cranial cavity by passing, first lateral and then dorsal to the *trabecula*.

AMIA CALVA

In *Amia* (Allis, '97, etc.) the myodome lodges the external rectus muscles and the pituitary veins, and corresponds to the dorsal compartment only of the myodome of Teleosts. It has no basioccipital extension, being limited to the subpituitary and prootic regions. The hypophysis and *saccus vasculosus*, both enclosed in the membranous pituitary sac, project ventrally into this myodomic cavity, the hypophysis lying immediately posterior to the presphenoid bolster and the *saccus* projecting posteriorly beneath the prootic bridge. The other rectus muscles have their origins on the lateral surface of the presphenoid bolster. The myodome has a large orbital opening, bounded laterally by the pedicel of the alisphenoid, and the *nervus profundus* and *vena jugularis* traverse this opening to enter the *trigemino-facialis* chamber, the *recti externi* traversing it to enter the myodome, and the oculomotor and trochlearis nerves traversing it to reach their primary foramina,

which lie in the membranous wall of the *cavum cerebrale cranii*. All of these structures thus pass mesial to the pedicel of the alisphenoid, as do also, morphologically, the pituitary vein and the abducens nerve. The *arteria carotis externa* and the *nervi maxillaris* and *mandibularis trigemini*, on the contrary, pass lateral to this pedicel to enter the trigemino-facialis chamber.

The trigemino-facialis chamber is not separated by a wall of bone into ganglionaris and jugularis parts, as in most of the Teleostei, and, because of the absence of a bony floor, the chamber is in direct communication with the myodomic cavity.

A ventral compartment of the myodome, as a functional myodomic cavity, is wholly wanting in this fish, but is represented in certain canals traversed by the internal carotid and efferent pseudobranchial arteries, the internal carotid artery of either side being accompanied, in part of its course through its canal, by the palatine branch of the facialis and the pharyngeal branch of the glossopharyngeus. These several canals were fully described in an earlier work (Allis, '97, p. 496) and were there called the palatine, internal carotid, and efferent pseudobranchial canals. The palatine canal of either side, as there described, lies between the parasphenoid and the ventrolateral surface of the chondrocranium, and the *nervus palatinus facialis* enters it at a certain distance anterior to its hind end, the posterior portion of the canal lodging only the internal carotid artery and the pharyngeal branch of the *nervus glossopharyngeus*. The internal carotid canal arises from this palatine canal and, running upward, traverses the cartilaginous presphenoid bolster to enter the *cavum cerebrale cranii*. The efferent pseudobranchial canal is in two sections, one of which traverses the lateral bounding wall of the orbital opening of the myodome, while the other penetrates the presphenoid bolster to fall into the internal carotid canal. My palatine canal is the *canalis parabasalis* of Gaupp's ('05 a) account of *Lacerta*, and conditions in other vertebrates, to be later considered, show that it should be considered as formed by the fusion of two canals, one traversed by the *nervus palatinus facialis* and the other by the internal carotid artery.

In the adult *Amia* the efferent pseudobranchial artery gives off its ophthalmica magna branch as it traverses the orbital opening of the myodome, the artery and this branch thus both appearing to here lie dorsal to the trabecula. In 8-mm. and 10-mm. embryos I, however, find the artery passing ventral to the trabecula and there falling into the internal carotid as it turns upward to pass between the trabeculae. From the artery so formed the arteria ophthalmica magna arises, and runs outward, dorsal to the trabecula, thus lying, in *Amia*, on the opposite side of the trabecula to that in which it is shown by Dohrn in a 10-mm. embryo of the trout (Dohrn, '86, fig. 2). The development of these arteries and their relations to the trabeculae need further investigation.

The prootic bridge, which forms the roof of the prootic portion of the myodome of the adult *Amia*, is of relatively late formation, for it is not found in a 40-mm. specimen. In a 43-mm specimen it has been formed, and, as in *Salmo*, lies at a certain distance dorsal to the fenestra ventralis myodomi and separated from the tip of the notochord by an open space, closed by membrane, which is the homologue of the fenestra basicranialis posterior of the Sauropsida. The saccus vasculosus lies, in this specimen, wholly anterior to the anterior edge of the prootic bridge, directly in line with it and embedded in the dorsal surface of loose stringy connective tissue which fills this posterior portion of the myodome. The recti externi, which, in the adult, extend to the hind end of the myodome, do not, at this age, extend posteriorly even as far as the hind end of the saccus vasculosus, having their origins approximately in the transverse plane of the posterior opening of the trigemino-facialis chamber.

From these conditions in *Amia*, it is evident that the pre-spinal and prootic portions of the normal teleostean myodome would arise if the cartilage which, in *Amia*, separates the myodome from the canals for the internal carotid and efferent pseudobranchial arteries were to be resorbed, leaving more or less developed membranes in its place. This cartilage is known to undergo resorption during the ontogenetic development of cer-

tain of the Teleostei (*Salmo*, *Gasterosteus*), and skeletogenous tissues capable of taking a membranous form would certainly be left in its place. The membranous tissues that would then represent the presphenoid bolster would not offer a firm point of attachment for the rectus muscles, and it would be wholly natural for certain of them to seek more solid points of origin, and one of them actually has, in most of the Teleostei, acquired such an origin by first creeping downward on to the dorsal surface of the parasphenoid and then pushing posteriorly in the open end of the persisting remnant of the palatine canal of my descriptions. This muscle actually is the rectus internus, but it is possible that it was primarily the rectus inferior, that muscle and the rectus internus undergoing an exchange of function and so giving rise to that manner of innervation of these muscles that I have described in several of these fishes (Allis, '03, '09), and which I now find to be apparently definitely related to the presence of a functional ventral myodomic compartment. Where that compartment is wanting, as in *Amiurus*, or present but non-functional, as in *Lepidosteus*, *Polypterus*, *Polyodon*, *Acipenser*, and higher vertebrates, these muscles are innervated approximately as they are in *Amia* (Allis, '08 b).

The definitive rectus internus of the Teleostei, in thus shifting its point of origin, passed dorsal to the efferent pseudobranchial artery and dorsolateral to the internal carotid. The membranous tissues representing the presphenoid bolster were then pressed together in the median line by these muscles, and became the median vertical myodomic membrane, the internal carotid arteries still being enclosed in it, in a membranous canal, the homologue of the cartilaginous canals of *Amia* fused to form a single canal. The floor of the myodome of *Amia* became the horizontal myodomic membrane, which becomes adherent to the ventral surface of the membranous pituitary sac and seems to end there. It, however, certainly continued, primarily, beyond that point and was continuous with the ventral end of the interorbital septum. The efferent pseudobranchial arteries were necessarily pressed ventrally by the recti interni, and, losing their connections with the internal carotids,

acquired a cross-commissural connection with each other. A basioccipital portion of the dorsal compartment of the teleostean myodome would then be added to the prootic portion, developed as above set forth, whenever an aortal groove similar to that in Hyodon had been developed and retained; and a posterior extension of the ventral compartment would be acquired by the recti interni pushing posteriorly between the floor of that compartment and the underlying parasphenoid. The many variations that I have described above in the myodome of the non-siluroid Teleostei would then all arise by different degrees of ossification of the several membranes in this region.

As already stated, the internal carotid arteries of *Amia* traverse the presphenoid bolster in order to enter the cavum cerebrale cranii, and, although the development of this bolster has not yet been worked out, there seems no question that it is formed by the hind ends of the trabeculae. The median vertical myodomic membrane of the Teleostei, which in those fishes represents the presphenoid bolster of *Amia*, would then also represent the hind ends of the trabeculae. The basisphenoid of the Teleostei cannot then be the exact homologue of the presphenoid bolster of *Amia*. The fenestra ventralis myodomus of the adult *Amia* lies posterior to the presphenoid bolster; it must then be bounded laterally by the so-called anterior prolongations of the parachordals, and hence correspond to Gaupp's fenestra basiscranialis posterior in embryos of *Salmo*, the fenestra hypophyseos of these embryos apparently being represented in the internal carotid canals of *Amia*. The fenestra hypophyseos is said by Gaupp ('05 b, p. 585) to be a persisting portion of the large fenestra basiscranialis anterior of early embryos, and it is said by him to be always traversed by the ectodermal stalk of the hypophysis. The hypophysis must then lie, in these early embryos, dorsal to this fenestra, and as the internal carotid arteries, in the Holostei and Teleostei, run upward anterior to the hypophysis, they must traverse the fenestra. The hypophysis must then have later shifted posteriorly to a position dorsal to the fenestra interparachordalis

(fenestra basicranialis posterior, Gaupp), leaving the carotid arteries behind it, in persisting remnants of the fenestra hypophyseos which I have called, in *Amia*, the internal carotid canals.

The carotid arteries do not, in either *Amia* or the Teleostei, enter any part of the dorsal myodomic cavity. In certain other fishes and in higher vertebrates they become included in that cavity. The arteries must accordingly there have either shifted posteriorly, with the hypophysis, out of the fenestra intertrabecularis into the fenestra interparachordalis, or the inner walls of the canals traversed by them in *Amia*, both the carotid canals through the presphenoid bolster and those parts of the parbasal canals which lodge those arteries, must have been resorbed, the canals thus being added to the dorsal myodomic cavity. The arteries would then lie dorsal to the cartilaginous floor of the myodomic cavity, instead of, as in *Amia*, ventral to it; their foramina would lie near the hind end of the subpituitary portion of the pituitary fossa, instead of anterior to it; and a part of the ventral compartment of the teleostean myodome would be added to the definitive myodomic cavity.

The septum interorbitale may now be considered, for it forms a direct anterior prolongation of the median vertical myodomic membrane and hence must be of similar origin. This septum is said by Gaupp ('05 b, p. 585) to characterize the tropibasic cranium, and to be found in many of the Selachii (Plagiostomi?), in the Ganoidei, the Teleostei, excepting the Siluridae and Homaloptera, and the Amniota. The platybasic cranium, in which this septum is wanting, is said to be found in many of the Selachii and in all of the Amphibia. In the Teleostei the septum is said to lie above the trabeculae (Gaupp, '05 b, pp. 667 and 762), between them and the cavum cerebrale cranii. The septum must then be formed by the ventral portions of the side walls of the primordial cranium pressed together in the median line, and this is in accord with Gaupp's conclusion in his work on *Lacerta*, where he says ('00, p. 553) that this septum must either be a wholly new formation of the tropibasic (tropidobasic) cranium or be formed from material derived from the side walls and floor of the platybasic (homalobasic) cranium,

and he definitely decides in favor of the latter supposition. Fuchs, however, decides just as definitely in favor of the first-mentioned supposition, for he says ('12, p. 104) that, in *Chelone*, the trabeculae take no part in the formation of the septum; that the septum is a new formation, peculiar to the tropibasic cranium; that it first appears as a keel-shaped outgrowth (*Vorwölbung*) on the ventral surface of the primordial basis cranii, and that it increases in height by growing upward. How a ridge on the ventral surface of the basis cranii could increase in height by growing upward is not at first quite clear, but in certain of the figures given by Fuchs the fundament of the septum is shown lying between the trabeculae, and hence capable of growing upward between them. This would of course leave the trabeculae near the ventral end of the septum, and this is the position in which they are shown in one of the figures given by Fuchs (*l. c.*, fig. 16 b). It is further said that, in later stages of development than that shown in the above-mentioned figure, the trabeculae are no longer recognizable in the optic region, but persist in the region of the hypophysis and from there run forward and fuse with the lower, thickened portion of the septum interorbitale. It is, however, particularly said by Fuchs that the ventral portions of the side walls of the cranium are here formed by the trabeculae, and that, in the embryo shown in his figures 16 a and 16 b, the trabeculae, in the region anterior to the nervus opticus, are reduced to connective tissue cords which lie near the upper end of the septum.

There is thus a difference of opinion as to the manner in which this septum arises, and there would also seem to be some confusion in Fuchs's statements regarding it. My own work leads me to suggest that the epichordal and hypochordal bands of skeletogenous material, known to be developed in the spinal region of embryos, are continued forward into the prespinal region, and that the trabeculae are there developed from them. These two morphologically distinct portions of the trabeculae are fused to form the basis cranii in the orbital region of the platybasic cranium, just as they are always fused, in embryos, to form the parachordal plate in the prootic region, and usually

so fused in the basioccipital region also. In the tropibasic cranium they have been forced apart, doubtless by pressure of the eyeballs, and the interorbital septum is formed from the material of the hypochordal bands and the tissues between them and the epichordal bands, the latter bands forming the floor and side walls of this part of the cranial cavity. The trabeculae might then be said by certain authors to lie at the ventral end of the interorbital septum, and by others to lie at its dorsal end. This would also explain how, in fishes where the interorbital and internasal septa are directly continuous with each other, the trabeculae are said by certain authors to form the ventral edge of the internasal septum, and by certain others to form its dorsal edge (Allis, '13).

LEPIDOSTEUS OSSEUS

In *Lepidosteus* there is no functional myodome, but the pre-existing spaces which correspond to both its dorsal and ventral compartments occur and were fully described by me in my work on the mail-cheeked fishes. The space representing the dorsal compartment lies, as does the functional myodome of *Amia*, dorsal to the cartilage which actually forms the basis cranii, the space that represents the ventral compartment lying ventral to that cartilage, between it and the underlying parasphenoid, and lodging, as in *Amia*, the internal carotid arteries and the palatine branches of the facialis nerves. Veit ('07), in a work that did not appear until after my own was sent to press, had previously described, in a 150-mm. specimen of this fish, the space representing the prootic portion of the dorsal compartment, calling it the *cavum saccivasculosi*, and he later ('11), described, in younger specimens, the development of the cartilages that bound that space.

In 8 to 16-mm. embryos of this fish, Veit ('11) says that the notochord is the only recognizable skeletal element; and it ends with a blunt point against the hind wall of the infundibulum, its extreme tip turning slightly ventrally. In embryos 10 to 11 mm. in length the notochord is in similar position, but three cartilaginous elements have now developed on either side of

the brain: a parachordal cartilage which extends from the transverse plane of the root of the nervus glossopharyngeus to that of the root of the nervus trigeminus; a polar (*Pol*) cartilage, which lies lateral to the anterior end of the notochord and extends, in a direct anterior prolongation of the line of the parachordal, from the root of the trigeminus to about the middle of the length of the hypophysis; and a trabecular cartilage, which, lying in the line prolonged of the other two cartilages, extends from about the middle of the length of the hypophysis to a point in front of the nervus opticus. The fundament of the musculus rectus externus of either side lies directly against the related polar cartilage.

In embryos of this fish 11 to 12-mm. in length the adjoining ends of the parachordal polar, and trabecular cartilages of either side have fused with each other to form a continuous cartilage, the part formed by the polar and trabecular cartilages lying, as shown in the figures, slightly dorsal to the level of the anterior end of the notochord. The trabeculae of opposite sides have fused with each other anterior to the recessus preopticus, thus enclosing a large fenestra basiscranialis, into the hind end of which the anterior end of the notochord projects. The polar cartilages now occupy the positions of the so-called anterior prolongations of the parachordals of Swinnerton's and Gaupp's descriptions of *Gasterosteus* and *Salmo*, and hence of the 'Balkenplatten' of Stöhr's descriptions of *Salmo*. The recti externi have now become inserted on the polar cartilages, and, because of this or for some other reason, the fenestra basiscranialis is there slightly constricted. The fenestra encloses the ventral portions of the infundibulum and recessus preopticus, and in later stages the hypophysis and saccus vasculosus come to lie, respectively, in the interpolar and interparachordal portions of it.

In embryos 14 to 20-mm. long the region under consideration has not changed in any important respect. The planum orbitonasale, formed by the fusion of the anterior ends of the trabeculae, begins immediately anterior to the recessus preopticus and extends forward beyond that part of the lamina terminalis

which forms part of the actual ventral surface of the brain, this lamina being bent at nearly a right angle and presenting surfaces that are the one actually ventral and the other anterior. A prootic bridge has not yet begun to be formed, but it is shown by Parker ('82) in somewhat older embryos, and is there at first separated from the otic portion of the parachordal basal plate by a large fenestra basicranialis posterior similar to the one in *Amia* and *Salmo*.

Veit does not give the relations of the internal carotid and efferent pseudobranchial arteries to the cartilages bounding the fenestra basicranialis of his descriptions, but in small embryos of this fish (size not given) I found ('09) the internal carotid running forward beneath the basis cranii, being there joined by the efferent pseudobranchial artery, and the artery so formed then turning upward through the fenestra basicranialis. Whether the part of the fenestra so traversed lies between the polar or trabecular cartilages cannot be definitely told by comparison with Veit's figures, but it would seem as if it must be between the hind ends of the trabeculae, the membranous pituitary sac lying dorsal to the polar cartilages. The conditions in this fish thus differ from those in the adult *Amia* only in that the efferent pseudobranchial artery does not traverse a foramen and canal in the cartilage of the basis cranii before falling into the internal carotid, and in that the recti externi have not invaded the myodomic space.

POLYPTERUS

In the neurocranium of the adult *Polypterus* there is a large pituitary fossa, the posterior portion of which is roofed by a horizontal bridge of the so-called sphenoid bone. The hind end of the pituitary body projects posteriorly beneath this bridge, and Waldschmidt ('87) shows it there surrounded by what he calls 'maschiges, fettartiges Gewebe.' This tissue apparently fills the space between the membranous pituitary sac and the walls of the cartilaginous pituitary fossa, the space thus corresponding to the prootic portion of the functional myodome of *Amia*. In his text figure 8, Waldschmidt shows the side wall of the

pituitary fossa perforated by a cord of tissue. It is not said what this cord of tissue is, but it is undoubtedly the pituitary vein described by me (Allis, '08a) in a 75-mm. specimen of *Polyp-terus senegalus*. This pituitary vein falls into a vein that I called the internal jugular, but which is more appropriately called the vena orbitalis inferior. This vein comes from the orbit, accompanied by the internal carotid artery and the nervus palatinus facialis, and after receiving the pituitary vein, is joined by a vein that I called the external jugular, but which is a vena orbitalis superior and is accompanied by the external carotid artery. The vein formed by the fusion of these two is the vena jugularis of the present descriptions. Running posteriorly, it traverses a short canal in the cartilaginous portion of the lateral wall of the chondrocranium, between the foramina by which the nervi trigeminus and facialis traverse that wall, and issues from the cranium, with the nervus facialis, at the hind edge of the ascending process of the parasphenoid. The external carotid artery unites with the internal carotid, and the artery so formed continues posteriorly in a canal through the ascending process of the parasphenoid, accompanied by a sympathetic nerve. At the hind end of this canal it receives the efferent artery of the hyoid arch, and then, becoming the lateral dorsal aorta, enters the aortal canal in the basioccipital, already referred to when describing the conditions in *Hyodon*.

The conditions in this fish are thus markedly different from, but nevertheless strictly homologous to, those in the other fishes so far considered. There is a dorsal myodomic cavity strictly similar to that in the *Holostei*, and a ventral compartment represented by the canals through the ascending processes of the parasphenoid. The median portion of the parasphenoid and the lateral walls of the canals through the ascending processes of that bone must then, together, correspond to the parasphenoid of *Amia*, the mesial walls of the latter canals corresponding to the ascending processes of the parasphenoid of *Amiurus*. The canal traversed by the vena jugularis, which lies partly in the lateral wall of the chondrocranium and partly between that wall and the lateral wall of the ascending process of the parasphenoid, is the pars jugularis of a trigemino-facialis chamber.

CHONDROSTEI

The descriptions that I find of the pituitary region of the cranium of the Chondrostei are incomplete, and but little can be said about it. A slight pituitary fossa is shown by Bridge ('79) in the chondrocranium of *Polyodon*, and both I ('11) and Danforth ('12) have described the arteries in this fish. In embryos of from 150-mm. to 170-mm. in length the internal carotid runs forward along the ventral surface of the neurocranium, at first ventral to a short lateral process of the parasphenoid, and then, anterior to that process, in a groove on the ventral surface of the lateral edge of the basis cranii, lateral to the lateral edge of the parasphenoid. The artery there becomes enclosed in dense fibrous tissues which are attached to the cranial wall, and while in the canal thus formed, it is joined by the *nervus palatinus facialis*, which issues from the cranial cavity through a special perforation of the cranial wall. The internal carotid then enters a canal in the cranial wall, receiving while in it, the efferent pseudobranchial artery, and then immediately gives off the *arteria ophthalmica magna*. A small pituitary vein is sent outward from the pituitary fossa, through a special foramen in the cranial wall, and falls into the *vena jugularis*. The *nervus abducens* traverses a short canal in the cartilage of the basis cranii and, issuing from it, apparently again lies in the *cavum cerebrale cranii*, from which it definitely issues with the main root of the *nervus trigeminus*.

There is thus evidently, in *Polyodon*, a subpituitary space corresponding to the myodomic cavity of *Amia*, but the conditions need further investigation. The ventral compartment of the teleostean myodome is represented in the canal of fibrous tissue traversed by the internal carotid artery and the *nervus palatinus facialis*, this apparently corresponding to the canal through the ascending process of the parasphenoid of *Polypterus*.

PLAGIOSTOMI

Gegenbaur ('72) describes, in the Selachii, a large pituitary fossa (Sattelgrube), which extends from the postclinoid wall (Satellehne) to a traverse presphenoid bolster (Praesphenoidvorsprung) which lies slightly anterior to the foramina optica, and is said to lodge the lobi inferiores anteriorly and the pituitary body posteriorly. The presphenoid bolster is said to vary greatly in importance in different species of the Selachii and to be wholly wanting in some of them, the Scylliidae being included among the latter. The pituitary fossa is, in certain of these fishes, everywhere lined with the dura mater, this membrane forming both the perichondrial lining of the fossa and the sac which encloses the pituitary body. In others of these fishes there is a deeper, posterior portion of the fossa, shut off from the cavum cerebrale cranii by a portion of the dura mater which extends dorsoposteriorly from its anterior edge to the summit of the postclinoid wall. This subdural portion of the fossa is said to be traversed by the arteria carotis interna (vordere Carotis), by a vein, and by a lymph canal which Gegenbaur calls the canalis transversus. When this subdural space is wanting, the canalis transversus and the internal carotid arteries are separately enclosed in the cartilage of the basis cranii.

Parker ('76) later described the conditions in *Scyllium canicula*, and in his figures of embryos of that fish he shows conditions in the pituitary region strictly similar to those described and figured by Gegenbaur in the adult of *Scyllium catulus*. In two figures of the adult, Parker, however, shows a small pituitary fossa which lodges the pituitary body and is separated from the so-called infundibulum by a tall preclinoid wall. I have heretofore always considered this condition in this fish to be either an abnormality in the particular specimen examined by Parker, or a condition due to great age, for Parker shows both the preclinoid and postclinoid walls strongly calcified. I have, however, now examined two small adults of this fish, and I find the canalis transversus of Gegenbaur's descriptions occupying exactly the position of Parker's pituitary fossa,

and it is unusually large in both these specimens. It therefore seems certain that, if Parker's figures and descriptions be not wholly wrong in this particular respect, the specimen examined by him must have been exceptional and abnormal.

In my work on *Mustelus* ('01), I found the *canalis transversus* of Gegenbaur's descriptions traversed by the pituitary veins, and not by a lymph vessel, and this was later confirmed by work on other *Selachii* (Allis, '14 a). In this latter work I found the deeper, posterior portion of Gegenbaur's descriptions of the pituitary fossa particularly well developed in *Chlamydoselachus*, and I said of it that it had "the appearance of being a somewhat separate and independent fossa." It is sub-pituitary, as well as subdural in position, and is filled with tissues that seem to be in part tough connective tissues and in part of a different character.

In all the *Selachii* I have examined or can find described, the internal carotid arteries always lie anteroventral to the pituitary veins, as they do in the *Teleostei* and *Holostei*, and they are always separated from those veins by either membrane or cartilage. They always either fuse with each other in the median line, or are there connected by cross-commissure, and this fusion of the arteries is certainly not due, as it apparently is in the *Teleostei*, to any pressure of the muscles of the eyeball. In *Heptanchus*, *Mustelus*, and *Acanthias* I found these arteries joined by the efferent pseudobranchial arteries, either while still in the cartilage of the *basis cranii* or while lying between that cartilage and the lining membrane of the *cavum cerebrale cranii*. The internal carotids of these fishes thus do not enter the *cavum cerebrale cranii* until after they have received the efferent pseudobranchial arteries, which perforate the side walls of the pituitary fossa slightly anterior to the internal carotid canals, approximately in the region between the hind ends of the *lobi inferiores* and the pituitary body. In *Chlamydoselachus* I found the internal carotids entering the *cavum cerebrale cranii* before they received the efferent pseudobranchial arteries, but I now think this may be an error. The *nervus palatinus facialis* does not, in any of these fishes, come into any relation

to any part of the pituitary fossa, running forward, after issuing from the cranial cavity, ventral to the chondrocranium.

The early development of the cartilages in this region of these fishes differs somewhat from that in the Teleostei and Holostei. According to Sewertzoff ('99), the trabeculae, when first formed, are independent cartilages, which lie oral, and hence morphologically ventral, to the hypophysis, and because of the marked cranial flexure at this stage of development, these cartilages lie ventral to the parachordal plate and perpendicular to it, slightly posterior to its anterior edge. In later stages of development the anterior portions of the trabeculae are said by him to fuse with each other, their hind ends still remaining separate, but having now fused with the ventral surface of the parachordal plate. An opening is thus enclosed between the trabeculae and the parachordal plate, and the hypophysis is said to traverse it. It is called by Sewertzoff the intertrabecular basal fontanelle, and, as shown by Parker in *Scyllium* ('76, fig. 6, pl. 35), has approximately the extent of the pituitary fossa of the adult fish. In later stages this large fontanelle is greatly reduced by progressive fusion of the trabeculae, both anterior (ventral) and posterior (dorsal) to the hypophysis, but Sewertzoff says that the hypophysis still projects through it, and he so shows it in transverse sections of embryos of *Acanthias* (*l. c.*, figs. 14 and 15, pl. 30). The stalk of the hypophysis is said to run forward from this point and to end blindly, and it apparently does not traverse the persisting portion of the basal fontanelle in *Acanthias*, but it does in *Pristiurus* (*l. c.*, figs. 23 to 25, pl. 31).

The course of the internal carotid and efferent pseudo-branchial arteries is not given by Sewertzoff, but the internal carotids must certainly have traversed the posterior portion of the large primitive fontanelle, and hence that part of that fontanelle which persists in the oldest embryos of *Acanthias* described by him. It would, however, seem as if they could not have traversed that part of the fontanelle that persists in *Pristiurus*, for that part lies considerably anterior to the hypophysis, between the hind edges of the fenestrae opticae (*l. c.*,

fig. 27, pl. 31), and hence at the anterior end of the pituitary fossa of the adult.

The pituitary veins, also, are not described by Sewertzoff, and neither they nor their foramina are indicated in his figures. They are, however, apparently shown by Baumgartner ('15) in sagittal sections through this region in embryos of *Acanthias*. In that author's figures 2 to 9, he shows a vessel ventral to the anterior end of the notochord, and morphologically posterior to the hypophysis. This vessel is not lettered in the figures, but it must certainly be a cross-section of the venous commissure formed by the pituitary veins. In Baumgartner's figure 9, it is shown lying between the parachordal plate above and a ventro-anteriorly directed process of cartilage that is apparently considered by Baumgartner to be of parachordal origin, but which must represent a section through that part of the trabecular cartilage of Sewertzoff's descriptions which is formed by the fusion of the trabeculae of opposite sides dorsal (posterior) to the hypophysis. This commissural vein would then pass dorsal to the trabeculae, as it normally should. The process shown by Baumgartner forms the posterior boundary of an opening between it and the hind end of the trabecular cartilage, and is hence the intertrabecular basal fontanelle of Sewertzoff's descriptions, and a vessel, possibly the internal carotid artery, is shown lying directly in it.

The so-called intertrabecular basal fontanelle of these embryos of the Selachii would then seem to correspond to the fused anterior and posterior basicranial fenestrae of *Salmo* and *Gasterosteus*, the definitive fenestra of *Pristiurus* corresponding to the fenestra hypophyseos of *Salmo* and *Gasterosteus*, and the definitive fenestra of *Acanthias* corresponding to the fenestra basicranialis posterior of those fishes. This latter fenestra is, as will be later shown, the fenestra hypophyseos of the *Dipnoi*, *Amphibia*, and *Sauropsida*, in which the internal carotid arteries traverse the fenestra along its posterior border, sometimes separated by a median cartilage called the intertrabecula.

Neither Sewertzoff nor Baumgartner describe polar cartilages in these fishes, but van Wijhe ('05) describes them in early

embryos of *Acanthias*, between the trabeculae and parachordals and primarily independent of those cartilages, thus corresponding to the hind ends of the trabeculae of Sewertzoff's descriptions, and apparently also to the median, ventro-anteriorly directed process of the parachordal of Baumgartner's description.

The conditions in these fishes thus show that chondrification has taken place to such an extent in the prootic and subpituitary regions that the dorsal compartment of the teleostean myodome has been reduced, either to canals traversed by the pituitary veins or to some part of a deeper, posterior portion of the pituitary fossa of the chondrocranium. The remainder of the deeper portion of the fossa represents an anterior extension of the dorsal myodomic cavity which has been developed in some relation to the enclosure of the internal carotid arteries in it. The subdural canals traversed by those arteries after they leave this subpituitary space evidently form anterior prolongations of it, and were they to be added to it, and the pituitary fossa reduced to the proportions in *Ceratodus* and higher vertebrates, the arteries would traverse a peripituitary space separated from the *cavum cerebrale cranii* by the *dura mater*. The conditions here thus seem to indicate that the fenestra hypophyseos of these fishes is the homologue of the fenestra interparachordalis of the *Holostei* and *Teleostei*, and not of the fenestra hypophyseos of those fishes. The foramina carotica of *Amia* and the *Selachii* are then not homologous.

No ventral myodomic cavity is found in these fishes, except as it may be represented in a part of the canals traversed by the internal carotid arteries. The cross-commissure between these arteries has a position which suggests that it may have been utilized, in the *Teleostei*, to form the cross-commissure between the efferent pseudobranchial arteries.

In certain of these fishes, a canal in the lateral wall of the chondrocranium, traversed by the vena jugularis, represents, as in *Polypterus*, a pars jugularis of a trigemino-facialis chamber (Allis, '14b).

In the *Batoidei*, the pituitary fossa, as shown in Gegenbaur's figures, is but slightly developed, but as he says that a canalis

transversus is found in these fishes, as in the Selachii, the conditions are probably strictly similar.

DIPNOI

In *Ceratodus*, the so-called *pars ascendens* of the anterior process of the palatoquadrate of Greil's ('13) descriptions forms the lateral wall of a space which, in an earlier work (Allis, '14 c), I showed to be the homologue of the trigemino-facialis chamber of the Holostei. In early embryos of *Ceratodus* this chamber has anterior and posterior openings which Greil calls, respectively, the foramen sphenoticum commune and the foramen praeoticum basicraniale. In older embryos the foramen sphenoticum commune becomes separated into four parts by bars of cartilage developed in the connective tissues surrounding the nerves and vessels which traverse the foramen. One of these parts, called by Greil the foramen sphenoticum majus, transmits all the branches of the nervi maxillo-mandibularis and lateralis trigemini and the vena and arteria temporalis, the latter artery being the carotis externa of my descriptions of other fishes. A second foramen, called the foramen sphenoticum minus, transmits the nervus profundus and the vena capitis media, this latter vein being also called the vena pterygoidea. A third foramen, called the foramen hypoticum, transmits the nervus oticus trigemini; the fourth foramen transmitting the nervus abducens. The posterior opening of the chamber, the foramen praeoticum basicraniale, does not undergo subdivision in the oldest embryos considered by Greil, and it is traversed by the nervus facialis, the ramus palatinus facialis, the arteria temporalis (carotis externa), and the vena capitis lateralis; the latter vein being a posterior continuation of the vena capitis media (pterygoidea), and the two together forming the vena jugularis of my descriptions of other fishes. The floor of the trigemino-facialis chamber is formed by the processus basalis of the palatoquadrate, and the palatinus facialis, after issuing through the posterior opening of the chamber, runs forward ventral to this floor, between it and the underlying parasphenoid.

In these embryos the hypophysis lies at the hind end of a large fenestra basicranialis, and even projects posteriorly slightly beyond and beneath the tip of the notochord. The fenestra basicranialis is bounded laterally by cartilages which Greil considers of trabecular origin, the parachordal cartilage not extending anteriorly beyond the tip of the notochord. A vena hypophyseos is said to arise in the neighborhood of the hypophysis and to issue from the cranial cavity through a foramen sphenolaterale, which lies dorsal to the trabecula and anterior to the foramen sphenoticum minus. This vein falls into the vena pterygoidea (jugularis), and although it is not said to be connected with its fellow of the opposite side by a cross-commissural vessel, it is certainly the pituitary vein of my descriptions. There is no indication, in the figures given, of a membrane separating this vein from the cavum cerebrale cranii, but this membrane must certainly exist, for it occurs in all other fishes so far considered.

In early embryos the arteria carotis interna is connected with its fellow of the opposite side by a cross-commissural vessel, immediately posterior to the hypophysis and immediately ventral to the tip of the notochord, but Greil says this cross-commissure has aborted in the oldest embryos examined by him. Anterior to this cross-commissure, the artery gives off an arteria palatina, which runs forward ventral and mesial to the trabecula. The artery itself then runs upward mesial to the trabecula of its side and is distributed mainly to the brain, one branch, however, the arteria orbitalis, being sent outward through the foramen sphenolaterale with the pituitary vein, and a second branch, the arteria ophthalmica sent outward with the nervus opticus through the foramen opticum. Before passing upward through the fenestra basicranialis, the internal carotids are said to lie between the ventral surface of the chondrocranium and the underlying parasphenoid.

In the adult, the large fenestra basicranialis of the embryo is shown entirely closed by cartilage in the median vertical sections given by Günther ('71), Huxley ('76), and Bing ('05), and each of these authors shows a deep pituitary fossa with

pronounced postelinoid and preclinoid walls. Bing says the hypophysis lies in the posterior portion of this fossa, the anterior portion being filled with arachnoidal tissue (*Arachnoideal-maschen*). The postelinoid wall is evidently formed by growth of the epichordal and hypochordal bands of parachordal cartilage said by Greil to enclose the tip of the notochord in embryos. The preclinoid wall had not begun to be developed in the oldest embryos described by Greil. No foramina leading into the pituitary fossa are shown or described by any of these three authors.

I find, in an old and somewhat dissected skull of this fish, a perforation of the cartilage of the basis cranii at the bottom of the posterior portion of the pituitary fossa, and it is closed by tough membrane. A small canal in the cartilage leads from either orbit to the edge of this membrane and must certainly have transmitted a vein which either traversed the membrane or passed dorsal to it, in order to reach and drain the hypophysis. The space traversed by this vein, wherever it may be, is a dorsal myodomic cavity. The internal carotid artery of either side passes internal to the parasphenoid, is there joined by the efferent pseudobranchial artery (mandibular aortic arch of Greil's descriptions), and then becomes embedded in the cartilage of the basis cranii and covered externally by membrane. The arteries of opposite sides are connected by a cross-commissural vessel which lies posterior to the median perforation in the floor of the pituitary fossa, the canal traversed by this cross-commissure representing part of a ventral myodomic cavity. Anterior to this cross-commissure each artery runs forward ventral to the pituitary vein, sends forward the *arteria palatina*, and then certainly enters the pituitary fossa through a foramen that I find lying anterolateral to the median perforation in the floor of the fossa, but, as my skull had been cleaned and the arteries removed, I cannot definitely establish this. If it traverse this foramen, as seems certain, it must enter and traverse that anterior portion of the pituitary fossa which Bing says is filled with arachnoidal tissue, this part of the fossa then representing the internal carotid canals of *Amia*, fused with each

other and become part of the pituitary fossa of the chondrocranium. Whether this part of the fossa is separated from the cavum cerebrale cranii by the dura mater or not cannot be told from my specimen, but comparison with other fishes and with higher vertebrates show that it must be.

There are thus, certainly, in this fish, both dorsal and ventral myodomic cavities, and the dorsal cavity has apparently fused with the prepituitary portions of the canals traversed by the internal carotid arteries to form a single peripituitary space similar to that found in higher vertebrates and represented in the cavernous and intercavernous sinuses of man, as will be explained later. The foramina carotica lie at the hind edge of the pituitary fossa, as they do in higher vertebrates. The cross-commissure connecting the internal carotids is evidently the homologue of the cross-commissure in the Selachii, and probably not the homologue of the anastomosis of the arteries of opposite sides in the Teleostei.

The bar of cartilage separating the foramina sphenoida majus and minus is the homologue of the pedicel of the alisphenoid of *Amia*, and if the anterior edge of this bar of cartilage were to grow forward so as to pass beyond the foramina for the pituitary vein and the oculomotorius and trochlearis nerves, it would give rise to the orbital opening of the myodome of *Amia*.

AMPHIBIA

In the Amphibia there apparently is no vein comparable to the pituitary vein of fishes, for I find no such vein described, and the pituitary region is said to be drained, in certain of these vertebrates, by branches of intracranial veins. It might be assumed that the myodomic conditions here were as in *Amiurus*, where the pituitary veins are also wanting, but it seems much more probable that the ventral processes of the prootics have here been wholly suppressed, and that the basis cranii corresponds to the roof of the dorsal compartment of the myodome of fishes, and hence represents the primary basis cranii. The course of the internal carotid artery in *Rana*, and that of the nervus abducens both in *Rana* and *Salamandra*, favor this interpretation.

The internal carotid artery of *Rana* is said by Gaupp ('93 b), p. 403) to pass upward, in early embryos, mesial to the trabecula of its side, but, because of enveloping growth of the trabecular cartilage, soon to become enclosed in a primary foramen caroticum. Having traversed this foramen and entered the cranial cavity, the artery gives off the *arteria carotis cerebialis* and then itself issues from the cranial cavity through the foramen oculomotorium as the *arteria ophthalmica*. In later stages, that part of the trabecula between the foramina caroticum and oculomotorium is resorbed, and the internal carotid is said then to lie in the orbit and to send its cerebral branch inward through the foramen oculomotorium. Comparing these conditions in *Rana* with those I have described in the Teleostei, it is evident that the primary foramen caroticum of *Rana* must lie in what corresponds to the floor of the *cavum cerebrale cranii* of *Amia* and the Teleostei, for that floor, alone, is continuous with that part of the cranial wall which is perforated by the foramen oculomotorium.

The *nervus abducens* of *Rana* is said by Gaupp to issue from the cranial cavity in the sheath of the *ramus orbitonasalis trigemini*, and to pass, with that nerve, under, and hence morphologically anterior to, the *processus ascendens quadrati*. In fishes the corresponding branch of the trigeminus (*nervus profundus*) passes mesial and anterior to the pedicel of the alisphenoid, and always lies dorsolateral to the myodome, never traversing it. Comparison of these conditions would accordingly indicate that the dorsal myodomic cavity is wanting in *Rana*. In *Salamandra*, Fuchs ('10) shows the *nervus abducens* perforating the basis cranii and then lying mesial to the *arteria carotis interna* in a canal between the basis cranii and the parasphenoid, the *nervus palatinus facialis* lying lateral to the *carotis interna*. The hypophysis lies in a perforation of the basis cranii, and even projects ventrally slightly beyond it, lying in a slight concavity on the dorsal surface of the parasphenoid. The dorsal myodomic cavity must accordingly be wholly suppressed here by failure of the ventral processes of the prootics to develop, the canal which lodges the internal carotid artery and the *nervus*

palatinus lying directly beneath the floor of the cavum cerebrale cranii. Thus this canal is, as Fuchs says, not the homologue of the canalis parabasalis of reptiles, and also not the homologue of that same canal in fishes.

The fenestra hypophyseos of the Amphibia is then the homologue of the pituitary opening of the brain case of fishes, and not of either the fenestra hypophyseos or the fenestra ventralis myodorus.

The antrum petrosum laterale of Drüner's ('01) descriptions of the Urodela represents some part of a trigemino-facialis chamber, and quite certainly its pars jugularis only (Allis, '14 d), the pars ganglionaris of the chamber then being enclosed within the cranial wall. The lateral wall of the pars jugularis of the chamber is formed by that part of the palatoquadrate terminating in the processus oticus, the processus ascendens quadrati, which is the homologue of the pedicel of the alisphenoid of fishes, forming the lateral wall of a space which corresponds to the orbital opening of the myodome of *Amia*. There, however, apparently is, in these vertebrates, no cartilage corresponding to the floor of that opening of *Amia*.

In the Anura the conditions are apparently similar to those in the Urodela, for, in embryos of *Rana*, Gaupp ('93 b) shows the trigemino-facialis ganglion lying within the chondrocranium.

REPTILIA

In my work on the mail-cheeked fishes, I came to the conclusion that there was, in the pituitary region of the chondrocranium of *Lacerta*, a 'space of uncertain dimensions' which corresponded to a part, if not the whole, of the myodome of fishes. This space was between the cartilaginous floor of the cranial cavity and a membrane which was assumed to overlie it and to form the actual floor of the cavum cerebrale cranii, but I could not then find this membrane described. It is, however, shown by Gaupp ('02, fig. 6, p. 172), well developed, in a figure of a cross-section through the prootic region of a 32-mm. embryo of *Lacerta*, and in the space between it and the cartilaginous basis cranii the hypophysis and the nervi abducentes are shown.

The foramina for the internal carotid arteries are cut in the section, lying in the floor of this space and separated from each other by a median piece of cartilage which lies at a slightly lower level than the cartilage on either side of it. The internal carotid arteries are shown lying ventral to the basis cranii, each artery accompanied by, and lying mesial to, the nervus palatinus facialis of its side. No parasphenoid bone is shown, but comparison with a figure of a 47-mm. embryo (Gaupp, '05 b, p. 763) shows that that bone lies ventral to the nerve and artery and forms the floor of the canalis parabasalis of Gaupp's later descriptions ('05 a, p. 292), this canal of *Lacerta* thus being the homologue of the palatine canal of my descriptions of *Amia*. The piece of cartilage between the foramina carotica is the intertrabecula of Fuchs's ('12) descriptions of *Chelone*, and, as it forms part of the floor of the little space here under consideration, it cannot be part of the crista sellaris, as the lettering in Gaupp's figure of the entire chondrocranium of *Lacerta* ('00, fig. 1) would lead one to suppose. The nervus abducens enters the space here under consideration by traversing a foramen which perforates the cartilage of the chondrocranium, lateral to the lateral end of the crista sellaris, and issues from it into the orbit.

No pituitary veins are shown in Gaupp's figure of a cross-section through this region in *Lacerta*, but in an earlier work ('93, p. 571) he fully describes them. A vein lies along each lateral surface of the middle lobe of the hypophysis and is connected with its fellow of the opposite side by several cross-commissures, the largest of which lies posterior to the hypophysis. From either end of this posterior cross-commissure an important vein leads into a large vein which drains the blood from the orbital venous sinus, and the vessel so formed falls posteriorly into the vena jugularis interna. These veins thus must traverse the space of uncertain dimensions mentioned in my earlier work, which is a dorsal myodomic cavity. The internal carotid arteries run upward through the fenestra hypophyseos, and then along the lateral surfaces of the lateral lobes of the hypophysis, lying, in their course, anteroventral to the pituitary veins.

A ventral myodomic cavity is represented in those parts of the canales parabasales posterior to the foramina carotica.

The antipterygoid is said by Gaupp ('00, pp. 541 and 542) to be the homologue of the ascending process of the quadrate of the Amphibia and to be wholly wanting in the cranium of mammals. The ala temporalis of the mammalian cranium is considered by him to be represented, in reptiles, by the processus basipterygoideus. Fuchs ('12, pp. 91 to 95), on the contrary, maintains that the antipterygoid (epipterygoid, Fuchs) is the homologue of the mammalian ala temporalis, and that the processus basipterygoideus is the homologue of the processus alaris of the ala temporalis. To explain the difference in the relations of the nervus maxillaris trigemini to the antipterygoid and ala temporalis, he assumes that the nerve has, in mammals, slipped over the top of the antipterygoid in early stages of development.

I formerly concluded ('14d) that the antipterygoid of *Lacerta* was the homologue of the pedicel of the alisphenoid of *Amia*, and the processus basipterygoideus the homologue of the floor of the orbital opening of the myodome of *Amia*. The pars ascendens of the quadrate formed the lateral wall of the post-trigeminal portion of a trigemino-facialis chamber, as in the Amphibia. My present work leads me to consider these conclusions correct, but to consider the trigemino-facialis chamber of these vertebrates to be the homologue of that chamber of *Ceratodus* and the Holostei, and not of the chamber of the Amphibia and Teleostei; for the lateral wall of the chondrocranium, both of *Lacerta* and *Crocodylus* (Shiino, '14), is certainly the primitive cranial wall and not the outer wall of a trigemino-facialis recess. The processus basitrabecularis of *Crocodylus* would then represent a part of the floor of that chamber, and the processus pterygoideus quadrati a part of its lateral wall.

The vena cardinalis anterior of *Lacerta* is said by Gaupp ('00, pp. 547 and 548) to run posteriorly dorsal to the processus basipterygoideus and then along the external surface of the chondrocranium, thus lying wholly external to that cranium. This is exactly as it should be under my interpretation of the conditions, for this vein is the vena jugularis of my descriptions of

fishes, and in *Amia* it enters the orbital opening of the myodome and then traverses the trigemino-facialis chamber, lying always external to the wall of the *cavum cerebrale cranii*. Gaupp considers this vein to be the homologue of the sinus cavernosus of mammals, and as that sinus is intracranial in position, he concludes that the space traversed by the vein in *Lacerta*, which is actually extracranial, has been added to the cranial cavity in mammals. The sinus cavernosus is, however, a branch of the *vena cardinalis anterior (capitis media)*, and not that vein itself, as will be later explained.

MAMMALIA

Properly to explain the conditions in mammals it is necessary first to consider the *ala temporalis*. This element of the cranial wall has been considered by many authors to have its homologue in the antipterygoid of reptiles, but Gaupp considers it, as stated above, the homologue of the *processus basipterygoideus* of those vertebrates. A well-recognized objection to its being the homologue of the antipterygoid of the *Reptilia* is that the *nervus maxillaris trigemini* (second branch of the trigeminus) lies posterior to that element of the reptilian cranium, but anterior to the *ala temporalis* of mammals. Gaupp accounts for this by saying that, because of the absence of an antipterygoid in mammals, there was no intervening skeletal element, and the nerve has simply joined the first branch of the trigeminus instead of remaining with the third. Other authors have suggested that the nerve has either cut through or slipped over the top of the antipterygoid, or simply, for some unknown reason, chosen a presumably more direct or advantageous course on the other side of it. My work leads me to quite a different conclusion, and I look for the homologue of the *ala temporalis* in a part of the lateral wall of the trigemino-facialis recess of fishes.

In all of the lower vertebrates there is apparently always either a trigemino-facialis chamber, a *pars ganglionaris* of that chamber (trigemino-facialis recess), or both *partes ganglionaris* and *jugal* separated from each other by a wall of bone. The outer

wall of the pars jugularis of this chamber of fishes, and the pedicel of the alisphenoid are represented, respectively, in the Amphibia by the otic and ascending processes of the quadrate, the latter process being the homologue of the antipterygoid of the Reptilia (Allis, '14 c). These two portions of the neurocranium of fishes are thus secondarily acquired additions to it, and one or the other, or even both of them, is frequently wanting. In the Selachii, the pars ganglionaris of the trigemino-facialis chamber may be separated, by a partition of membrane or cartilage, into trigeminus and facialis portions, the latter portion then fusing with an acusticus recess to form an acustico-facialis recess.

Assume that, in a piscine skull, the pedicel of the alisphenoid and the lateral wall of the pars jugularis of the trigemino-facialis chamber are both wanting, as is actually the case in certain of the Teleostei; that independent trigeminus and acustico-facialis recesses have been formed, as in certain of the Selachii; that the muscles of the eyeballs have not acquired entrance into the preexisting myodomic cavities, as in many fishes; that these cavities have been reduced to the conditions found in *Ceratodus*; and that the trigeminus recess has been enlarged to such an extent that its floor projects ventrally below the level of the pituitary fossa (*sella turcica*), as it actually does in many of the Mammalia. If the wall separating the trigeminus and acustico-facialis recesses were then to be perforated, the facialis portion of the latter would be in communication with the trigeminus recess, and conditions would arise similar to those described by Voit ('09) in rabbit embryos, where the *cavum epiptericum* (trigeminus recess) and the *cavum supracochleare* (facialis recess) form a continuous cavity which communicates with the *meatus acusticus internus* (acusticus recess) through a *foramen faciale primitivum*. The facialis nerve would then issue from the facialis portion of this continuous cavity through a *foramen faciale secundarium*, the profundus nerve (first branch of the trigeminus) and trigeminus issuing from the trigeminus portion of the cavity, and their foramina of exit lying at the hind end of the orbit and not far from the foramina of the pituitary vein

and the oculomotorius, trochlearis, and abducens nerves. In the Teleostei and Selachii these several last mentioned foramina may lie relatively close together, and the chondrification or ossification of the tissues of the cranial wall may actually give rise to marked variations in the number and arrangement of the definitive foramina. Assume that the tissues surrounding the nervi maxillaris and mandibularis trigemini, as they issue from the trigeminus recess, chondrify to form a vertical bar of cartilage; that this bar grows forward so as to shut in the other foramina mentioned above, as the pedicel of the alisphenoid actually does in *Amia*; and that the tissues separating these other foramina from each other and from the nervus maxillaris persist as membrane. This would give rise, in this hypothetical cranium, to three fenestrations of the cranial wall which would be strictly similar, so far as the nerves traversing them are concerned, to the fissura orbitalis superior and the foramina ovale and faciale secundarium of Voit's description of embryos of the rabbit. If, then, the venous and arterial vessels of the region also have the same relations to these foramina that they do to the foramina in the rabbit, there would seem to be no reasonable doubt that the foramina, and hence their bounding walls, are strictly homologous.

In fishes the vena jugularis always runs posteriorly mesial to the pedicel of the alisphenoid, when the pedicel exists, and then always traverses the pars jugularis of the trigemino-facialis chamber, when it is present and independent of the pars ganglionaris. When the pars jugularis of the chamber is wanting, the vein passes along the lateral wall of the neurocranium, whether that wall be formed by the primary wall of the cranial cavity or by the lateral wall of a trigemino-facialis recess, never entering either the recess or the cavum cerebrale cranii. The pituitary vein arises from this vena jugularis and perforates the cranial wall, anterior to the trigemino-facialis chamber, to enter the dorsal myodomic cavity, never itself entering either the cavum cerebrale cranii or any part of the trigemino-facialis chamber. A branch is, however, sent into the cavum cerebrale cranii to drain the hypophysis, and, in certain Teleostei, this branch

is connected with an intracranial vein, the encephalic vein of Allen ('05), which enters the trigemino-facialis recess, perforates its lateral wall posterior to the nervus trigeminus, and falls into the vena jugularis. In other Teleostei the pituitary vein is connected with intracranial veins which issue through the foramen vagum there to fall into the vena jugularis. If either of these two connections were to become important, the flow of blood in the pituitary vein would be reversed, and a vein would be formed which would drain the hypophysial region and would issue, in the one case, through a foramen jugulare spurium, and, in the other, through a foramen jugulare.

In the Amphibia and Reptilia the vena jugularis always passes mesial to the ascending process of the palatoquadrate, or its homologue, the antipterygoid, and then, in each case, traverses the pars jugularis of the trigemino-facialis chamber, never there traversing any portion of the lateral wall of the neurocranium.

The arteria carotis externa of fishes, like the vena jugularis, always traverses the pars jugularis of the trigemino-facialis chamber, when that part of the chamber has been separated from the pars ganglionaris, never traversing the pars ganglionaris. On issuing from the chamber into the orbit, it always runs outward, posterior and lateral to the pedicel of the alisphenoid. In the Amphibia and Reptilia it traverses the pars jugularis of the trigemino-facialis chamber, always lying lateral to the lateral wall of the neurocranium, and issues from the chamber, posterior to the ascending process of the palatoquadrate in the Amphibia, or to the antipterygoid in the Reptilia, thus lying lateral to that element of the cranial wall.

In embryos of the porpoise the vena jugularis of fishes is represented in the vena capitis media plus the vena capitis lateralis, and, as described by Salzer ('95), all the cerebral veins empty into it, some anterior, some posterior to the nervus trigeminus, between it and the nervus facialis, and some in the region of the nervus vagus. The anterior of these three connections with the primitive vena jugularis loses its importance in later stages of development, the other two increasing, but varying in relative importance at different stages of development, and appar-

ently also in different species of the Mammalia, this giving rise to a vena jugularis interna which issues, either through a foramen jugulare spurium or a foramen jugulare, or even through both those foramina; these two connections with the primitive vein thus evidently corresponding to those referred to above in the Teleostei. The sinus cavernosus is said by Salzer (l. c., p. 252) to be formed from the veins which primarily collected the blood from the eyeball and the orbit, and which acquire a secondary connection with the sinus petrosus. This secondary connection must certainly be formed by a vein, the homologue of the pituitary vein of fishes, which has become important because of the abortion of the short vertical venous commissure which primarily connected the venae capites media and lateralis between the trigeminus and facialis ganglia. I do not find that Salzer mentions the abortion of this connection, but his figures show that it is absent in older embryos. Thus the sinus cavernosus of mammals is the pituitary vein of fishes, and it is said by Salzer (l. c., p. 242) primarily to have delivered the blood from the orbital veins into the sinus petrosus. Later, the flow of blood is reversed, in the porpoise, and the sinus cavernosus and the orbital veins are drained by the facial vein, the flow of the blood in the sinus cavernosus thus now being in the same direction as in the pituitary vein of fishes.

The sinus cavernosus of mammals thus certainly contains no part of the primitive vena jugularis, but a persisting portion of that vein forms the connection between it and the orbital veins. In the Sauria the sinus cavernosus is said by Grosser and Brezina ('95, p. 323) to be perhaps a remnant of the vena cardinalis anterior, and there to be extracranial in position (l. c., p. 321); neither of which statements is correct, for the conditions are here certainly as in the Mammalia. Gaupp ('00, p. 548) quotes Grosser and Brezina as here saying that the sinus cavernosus is actually (wohl) a part of the vena cardinalis, and adds that he has himself confirmed this, as well as its extracranial position, in embryos of *Chelone*.

These statements regarding this sinus led me formerly to conclude (Allis, '09, p. 193) that the venous vessel which tra-

verses the sinus cavernosus of man was the homologue of the vena jugularis of fishes; that the intercavernous sinuses represented the pituitary veins of fishes; and that the cavernous and intercavernous sinuses and the cava Meckelii together represented the myodome of *Amia* together with its so-called upper lateral, or trigemino-facialis chamber. This is, however, an error, for the so-called cavernous and intercavernous sinuses together represent a dorsal myodomic cavity plus the internal carotid canals, and the venous vessels traversing this cavity are, together, the homologues simply of the pituitary veins of fishes. The cavum Meckelii is then simply a trigeminus recess and not a trigemino-facialis chamber.

In Thane's figure ('94, fig. 405, p. 523) of a transverse section through the sinus cavernosus of the adult man, the outer wall of the sinus, formed by the dura mater, is thickened and is traversed by the oculomotorius, trochlearis, profundus (first branch of the trigeminus), abducens and maxillaris trigemini nerves. The inner wall of the sinus is continued across the dorsal surface of the sella turcica, and is there separated by a narrow space from the membranous pituitary sac, this space being traversed, on either side of that sac, by the intercavernous sinuses. The internal carotid artery enters this sinus through the inner part of the foramen lacerum, runs forward in the carotid groove on the lateral surface of the body of the sphenoid, and turns upward in a semicircular notch on the posterior surface of the preclinoid wall, this notch representing a remnant of the internal carotid canal of *Amia*. The artery lies lateral to the pituitary vein, but if the myodomic cavity were convex on its ventral surface, as it is in fishes, instead of concave, as in man, the artery would lie ventral and internal to the loop formed by the veins of opposite sides, as it does in fishes. The external carotid artery lies everywhere external to the cranial wall, as does also the vena jugularis externa, terminal branches only being sent into the cranial cavity.

The relations of the veins and arteries of man to the crania wall are thus, like those of the nerves, strictly similar to those in the hypothetical piscine cranium here under consideration,

and no suppositions have been made in regard to the latter that are not warranted by conditions actually found in fishes, excepting only the formation of a bar of cartilage between the nervi maxillaris and mandibularis trigemini and the fusion of the foramen for the nervus maxillaris with certain other foramina to form a single large fenestra; and, as already stated, marked variations in the fusions and groupings of the foramina in this region are of constant occurrence in fishes. It thus seems certain that the foramina in this region in the two crania are homologous, and it follows that the lamina ascendens of the ala temporalis of mammals is a bar of cartilage formed between the nervi maxillaris and mandibularis trigemini as they issue from a trigemino-facialis recess, and this element of the cranium is apparently characteristic of these vertebrates. The processus alaris of the ala temporalis must then be represented in some ventral portion of the basicapsular commissures of fishes, and apparently in that part which, in *Amia*, lies between the palatine foramen and the floor of the orbital opening of the myodome. If it includes the latter floor, it must include the processus basipterygoideus of reptiles, which seems improbable.

Certain other features of the region, which favor this interpretation of the conditions, may now be considered.

The myodomic cavity of the mammalian cranium, corresponding to the so-called cavernous and intercavernous sinuses of man, must necessarily extend, on either side, beyond the lateral edge of the foramen caroticum, and its roof is thus formed by what Terry ('17) has recently described as the spreading basal portion of his membrana limitans. The carotid foramen accordingly lies in the floor of this myodomic cavity and not, as Voit ('09) concluded was the case in rabbit embryos, in the floor of the cavum epiptericum. The nervus petrosus superficialis major (nervus palatinus facialis) of the rabbit does, however, perforate the floor of the cavum epiptericum, as Voit concluded, this being in accord with its course in the Teleostei, where it usually perforates the floor of the pars ganglionaris of the trigemino-facialis chamber, but may occasionally perforate the floor of the pars jugularis.

The *arteria carotis interna* of the rabbit is said by Voit to run upward through the foramen caroticum into the *cavum epiptericum*, which, as explained above, is certainly incorrect. The artery is said then to run forward dorsal to the *processus alaris* of the *ala temporalis*, which is in accord with my interpretation of the conditions, for that process forms part of the floor of the dorsal myodomic cavity. The artery is said by Voit to lie lateral to a cartilage 'c,' which Voit considers to form part of the lateral wall of the *cavum cerebrale cranii*. This cartilage is, however, certainly a chondrification of a membrane shown, in one of Arai's figures of this animal ('07, fig. 6, p. 432), running upward between the *hypophysis* mesially and the *arteria carotis interna* and the pituitary vein (so-called *sinus cavernosus*) laterally. This membrane is continued mesially between the *hypophysis* and the dorsal surface of the *sella turcica*, and is shown as a single membrane, but it must necessarily be formed by the fusion of two membranes, one forming the floor and the other the roof of the subpituitary myodomic cavity. The cartilage 'c' is evidently a chondrification of some part of this membrane, and may therefore represent a chondrification of either one of its two components; and its position and its coalescence with the floor of the *sella turcica* seem to indicate that its basal portion belongs to both membranes while its dorsal portion belongs to the dorsal membrane only and forms part of the roof of the myodomic cavity and hence of the wall of the *cavum cerebrale cranii*. The internal carotid accordingly here lies in a lateral portion of the myodomic cavity which has been separated from the median portion of the cavity by this wall of cartilage. The cartilages 'a' and 'b' of Voit are, as he concluded, remnants of the mesial wall of the *cavum epiptericum* (*trigeminus recess*).

Because of the passage of the internal carotid through what Voit considered to be a part of the *cavum epiptericum*, he concludes ('09, p. 551) that this artery of the rabbit, and hence also that of others of the *Mammalia ditremata*, must run upward lateral to the *trabecula*, the internal carotid of these animals thus not being the homologue of the similarly named artery of

the Mammalia monotremata and of lower vertebrates. Gaupp had previously suggested that the trabecula had here simply 'cut through' the artery, but Voit is not inclined to accept this suggestion. The bounding walls of this foramen are, however, under my interpretation of the conditions, of parachordal (polar) and not of trabecular origin, and there is, accordingly, no question here of its lying on one side or the other of the trabecula. It does, however, apparently lie lateral to the polar cartilage, and hence morphologically lateral, instead of mesial, to the trabecula, and a possible explanation of this will be given when the polar cartilages are considered later.

The processus pterygoideus arises from the ala temporalis at the base of its lamina ascendens, and hence, under my interpretation of the conditions, from the ventral edge of the lateral wall of the trigemino-facialis recess. Its position, alone, thus indicates that it is a remnant of the lateral wall of the pars jugularis of a trigemino-facialis chamber, and its relations to the nerves, arteries, and veins are in accord with this conclusion. The several branches of the nervus trigeminus all lie dorsal to it, as they should; the nervus petrosus superficialis major runs forward ventral to it; and the vena capitis media of embryos must necessarily have passed dorsal to the place where the process later develops, for that vein lies directly ventral to the nervus trigeminus. The relations of the arteria maxillaris interna (carotis externa of fishes) to the process vary. In embryos of the rabbit the artery perforates the process (Voit). In embryos of the dog it is said by Olmstead ('11) to traverse a canalis alaris s. alisphenoideum, which begins on the external surface of the lamina ascendens of the ala temporalis and issues on its anterior edge. The foramen rotundum opens into this canal, and the second branch of the trigeminus, passing through this foramen, enters the canalis alaris and, accompanying the arteria maxillaris interna, issues through its anterior opening into the orbit. In *Vespertilio* the artery is said by Grosser ('01) to enter the cranial cavity through the foramen ovale, then to run forward ventral to the second branch of the trigeminus, and to issue from the cranial cavity through an opening which corresponds to the

fissura orbitalis superior of man plus the foramina rotundum and opticum. In the Macrochiroptera the artery traverses a canalis pterygoideus in the basis cranii of this region (Grosser), while in *Rhinolopas* it lies, as in man, wholly free along the lateral wall of the cranium. This is, then, wholly in accord with the varying relations of this artery to the lateral wall of the cranium in fishes, the artery traversing the pars jugularis of the trigemino-facialis chamber in all of the Teleostei in which that part of the chamber occurs, traversing a foramen in its lateral wall in *Amia*, entering it with the nervus palatinus facialis in *Lepidosteus*, and lying wholly external to the lateral wall of the cranium in those fishes (*Cottus*, *Amiurus*) in which the pars jugularis of the trigemino-facialis chamber is not enclosed. It is thus evident that, both in the dog and in the Macrochiroptera, the processus pterygoideus has fused with the lamina ascendens of the ala temporalis and so has enclosed the external carotid in a canal which corresponds to a part of the pars jugularis of a trigemino-facialis chamber, and that, in *Vespertilio*, the mesial wall of this canal has been resorbed, the artery then lying in a part of a trigemino-facialis chamber.

The fovea epitympanica of rabbit embryos is a depression on the lateral surface of the chondrocranium, said by Voit ('09, p. 450) to lie between the crista facialis and the tegmen tympani. The tegmen tympani is said to arch over the upper edge of the fovea, and it is so shown in his figures, the tegmen apparently forming the dorsal portion of the lateral wall to the fovea. It is, however, said (*l. c.*, p. 449) that the tegmen is perforated by the foramen faciale externum s. secundarium, but as that foramen lies in the plane of the mesial wall of the fovea epitympanica, it would seem as if there must be some error in the descriptions. But however this may be, the fovea lodges the upper ends of the malleus and incus, and these two cartilages lie external to the nervus facialis, to the posttrigeminus portion of the vena capitis lateralis, and to the arteria stapediales (maxillaris interna, carotis externa). The fovea and the space traversed by this nerve, vein, and artery thus together form a cavity which has the relations to the cranial wall of the pars jugularis of a tri-

gemino-facialis chamber, the tympanic cavity of mammals thus being a derivative of this chamber of fishes. The tegmen tympani and the malleus, incus, and stapes are then quite certainly parts of the outer wall of this cavity, and hence derived from the quadrate, this being as Drüner ('04) has maintained for the malleus, incus, and stapes. It would also seem as if the annulus tympanicus must have the same origin, thus completing the outer wall of the cavity and encircling the part that was broken up to form the auditory ossicles. The fact that the stapes may be traversed by the arteria stapediales is in accord with the perforation, in *Amia*, of the lateral wall of the trigemino-facialis chamber by the external carotid.¹

The tympanic cavity is traversed, in mammals, by the chorda tympani, and Jacobson's nerve and sympathetic fibers enter it. In fishes the pars jugularis of the trigemino-facialis chamber is traversed by a sympathetic nerve and frequently (always ?) also by a communicating branch from the nervus facialis to the nervus trigeminus, and Jacobson's nerve enters it as a part of the truncus facialis. The communicating branch from the nervus facialis to the nervus trigeminus must then be the chorda tympani, and that nerve must be a prespiracular one, for in fishes it certainly is prespiracular. The chorda tympani must then be represented, in fishes, in the ramus mandibularis internus trigemini of my descriptions of *Amia* (Allis, '01, p. 188).

In fishes the spiracular canal or a diverticulum of it may lie along the lateral wall of the trigemino-facialis chamber. If a diverticulum of either of those canals were to expand into the pars jugularis of the chamber, it would evidently give rise to a tympanic cavity connected with the pharynx by an eustachian tube, or the same result would be obtained by the expansion in-

¹ Later work has somewhat modified this opinion and convinced me that the incus, alone, corresponds to the lateral wall of the trigemino-facialis chamber of fishes, both structures being derived from the posterior branchial-ray bar of the mandibular arch. The malleus and the teleostean quadrate both represent the epal element of the mandibular arch. The styloid and mastoid processes are, respectively, the anterior and posterior branchial-ray bars of the hyal arch, and the stapes probably the pharyngohyal. The chorda tympani is a posttrematic nerve.

to the chamber of a diverticulum of a plica hyomandibularis (Drüner, '03).

The conditions in *Echidna* remain to be considered. In an earlier work (Allis, '14 b) I came to the conclusion that the cavum epiptericum of Gaupp's descriptions of embryos of this animal was the strict equivalent of the trigemino-facialis chamber of *Amia* less its pars facialis, this conclusion being based on my interpretation of Gaupp's descriptions of the venous vessels of the region. According to him ('08, p. 598), there is, in the cavum cerebrale cranii of this animal, a large cross-commisural venous vessel, anterior to the hypophysis and issuing on either side through the fenestra pseudo-optica into the cavum epiptericum. There, one part of this vessel turns forward and passes into the orbit, the other turning posteriorly in the cavum epiptericum and becoming the sinus cavernosus. This so-called sinus cavernosus is said to pass ventral to the ganglion trigeminum, and it is shown, in a figure of a transverse section through this region, lying ventrolateral to the base of the taenia clino-orbitalis, the hypophysis lying mesial to the taenia. Posterior to this point, and hence apparently posterior to the sella turcica, the sinus cavernosus turns laterally and falls into the sinus transversus, the latter sinus descending almost vertically in front of the otic capsule. The fusion of these two veins is said to form the vena capitis lateralis, which issues from the cranial cavity through the hindermost corner of the fenestra sphenoparietalis and immediately enters the sulcus facialis on the external surface of the chondrocranium. In a slightly older embryo the sinus cavernosus is said (*l. c.*, p. 629) still to be connected with the sinus transversus, but to be now also prolonged posteriorly as the sinus petrobasilaris, which runs posteriorly in the cavum cerebrale cranii, sends a branch outward through the foramen jugulare, and then itself issues through the foramen occipitale magnum.

From these descriptions I concluded ('14 b) that the so-called sinus cavernosus, plus the vena capitis lateralis, must form a vein the homologue of the vena jugularis of fishes. That vein could not then enter the cavum cerebrale cranii, as Gaupp says it does,

and I concluded that there must be some error in the descriptions, for I did not question the identification of the veins. Because of the position of this vein, I concluded that the cavum epiptericum was a trigemino-facialis chamber. This is, however, wrong, for the so-called sinus cavernosus is, in reality, the homologue of the pituitary vein of fishes, and not of the vena jugularis. This vein of *Echidna* must then traverse a myodomic cavity, as it does in the *Mammalia ditremata*, and there must be a membrane separating it from the cavum epiptericum, that membrane being a part of the membrana limitans of Terry's ('17) descriptions of the cat and forming the roof of a myodomic cavity which is the sinus cavernosus properly so-called. The pituitary vein then traverses this cavity, as it does in man, and that part of the so-called sinus which Gaupp says turns laterally and falls into the sinus transversus, is the vena encephalica of fishes, this latter vein falling into the vena capitis lateralis (vena jugularis of fishes) after and not before, it issues from the cavum epiptericum. The vena capitis lateralis has here, as in the *Mammalia ditremata*, lost its primitive continuity with the vena capitis media; the persisting portions of these veins both lie external to the cranial wall; and the cavum epiptericum is a trigemino-facialis recess. The conditions in this animal are then strictly similar to those in the *Mammalia ditremata* except that a taenia clino-orbitalis has been formed, comparable to, but somewhat different from, the cartilage 'c' of Voit's descriptions of the rabbit.

In the adult *Echidna* it would seem, from Gaupp's descriptions, as if certain of the bones forming the lateral wall of the cranium were developed in the lateral wall of the cavum epiptericum (trigeminus recess), and certain of them in the lateral wall of the pars jugularis of a trigemino-facialis chamber, for certain of the bones are said (*l. c.*, p. 650) to be ossifications of the membrana speno-obturatoria, which is said to lie external to the ala temporalis. The taenia clino-orbitalis is said by Gaupp (*l. c.*, p. 647) to have fused, in the adult, with the lateral edge of the sella turcica along the full length of the sella, the fissura pseudo-optica thus being greatly reduced in size; the

development of this bar of cartilage doubtless accounting for the suppression of a posthypophysial commissure between the pituitary veins of opposite sides of the head.

The so-called parasphenoid of *Echidna* is considered by Gaupp ('05 a) to be the homologue of the ascending process of the parasphenoid of the *Sauria*, and also of the mammalian pterygoid, the latter bone not being the homologue of the pterygoid of reptiles. The bone of *Echidna* is said to lie, in embryos, directly upon the cartilage of the basis cranii, without intervening connective tissue, and later to fuse with the sphenoid (Keilbein) as part of its processus pterygoideus. No cartilage has been found in this bone in *Echidna*, but it is said to be found in the pterygoid of mammals. The bone lies anterior to the foramen caroticum, the internal carotid arteries accordingly not coming into any relations to it. The nervus parabasalis (palatinus facialis) is said to run forward external to the posterior portion of the bone, but, anterior to the point of exit of the nervus opticus from the cranial cavity, it perforates the bone through a foramen parabasale, and so enters the anterior portion of the cavum epiptericum. There is thus no canalis parabasalis in this animal, and the relations of the parasphenoid to the chondrocranium, to the internal carotid arteries, and to the ramus palatinus facialis all show that it corresponds to the ascending process of the parasphenoid of *Amiurus*, and to the mesial plate of that process of the parasphenoid of *Polyp-terus*, and that it is accordingly an ossification in the roof of a ventral myodomic cavity and not in its floor.

CARTILAGINES POLARIS AND ACROCHORDALIS

Polar cartilages were, as already stated when describing the *Selachii*, first described by van Wijhe ('05) in embryos of *Acanthias*, where the cartilage of either side is said by him to lie between the trabecular and parachordal cartilages, but it soon fuses with both those cartilages and then forms, with the trabecula, the ventral border of the orbital fenestra. The posterior border of the orbital fenestra is said to be formed by the lamina antotica, which is an outgrowth of the anterior end of

the parachordal of its side. Nothing is said of the relations of the eye-muscles, arteries, and veins to these cartilages. Sewertzoff ('97, '99), in his work on embryos of this same fish (*Acanthias*), did not find these cartilages, and he says that the alisphenoid, which is van Wijhe's *antotica*, is primarily a wholly independent cartilage and hence not an outgrowth of the parachordal. He considers it to be a prechordal structure, and says that it is apparently developed in close relations to the eye-muscles, the four rectus muscles and the obliquus superior all having their insertions on it. The only other fish in which this cartilage has been described is, so far as I know, *Lepidosteus*, where it has been described by Veit and has been already referred to when considering that fish. The cartilage is there said to give insertion to the rectus externus, this cartilage of this fish thus apparently corresponding, functionally, to the base of the alisphenoid cartilage of Sewertzoff's descriptions of *Acanthias*, as it does also to the eye stalk of the adult selachian. A polar cartilage, although only described in these two fishes, has been recognized and described in certain of the Sauropsida and Mammalia.

In 5-mm. chick embryos and 8 to 9-mm. embryos of the duck, Sonies ('07) finds no cartilage as yet developed in the cranial region. The notochord is said to extend far up in the plica encephali ventralis, and its tip is there bent slightly ventrally and is lost in connective tissues behind the hypophysis. In slightly older stages, an unpaired cartilage, the cartilago acrochordalis, develops around the anterior end of the notochord, the cartilage inclining dorso-anteriorly and the notochord perforating it from its dorsal surface. The parachordals are said to then develop, posterior to this acrochordalis cartilage, as a simple unpaired median plate, for, although always thickest along their lateral edges, they are always continuous with each other dorsal to the notochord and, in most instances, also continuous ventral to it. These two primarily independent and unpaired cartilaginous plates, the acrochordalis and parachordalis, then become connected with each other, on either side, by a short cartilage which is called the cartilago basiotica, these

two paired cartilages developing as independent pieces in the duck, but in the chick in continuity with the anterior edge of the parachordal plate. The basal plate is thus completed, and it is perforated by a median space, traversed longitudinally by the notochord, which is said to be the fenestra basicranialis posterior and which has the position of that fenestra in *Lacerta* and the *Amphibia*.

The trabeculae appear as independent paired cartilages at about the same time as the cartilagine basioticae, lying rostral to the nervi optici and nearly at right angles to the basal plate. An independent cartilago polaris then develops, in the duck, on either side of the hypophysis, between the trabeculae and the ventral surface of the basal plate, and later fuses with both of those cartilages, usually first with the trabeculae, but occasionally first with the basal plate. In the chick the cartilago polaris is, from the very first, continuous with the hinder end of the trabecula of its side. The fusion of the polar cartilages with the basal plate takes place in the line of the fusion of the cartilagine acrochordalis and basioticae, and a fenestra hypophyseos is thus enclosed, which lies nearly at a right angle to the fenestra basicranialis posterior and is separated from it by the cartilago acrochordalis (*l. c.*, p. 426). The side walls of this fenestra hypophyseos are at first formed both by the polar cartilages and the hinder ends of the trabeculae, but, as the trabeculae gradually fuse with each other in the median line, that part of the fenestra which was primarily enclosed between them is gradually suppressed, the trabeculae then only forming its anterior wall, the tuberculum sellae. The cartilago acrochordalis, projecting dorso-anteriorly, is said to form the dorsum sellae. A processus infrapolaris develops later on either side, from the posteroventral surface of the polar cartilage, and in *Sterna* projects posteriorly beneath and parallel to the basal plate, its hind end fusing with it on either side of the fenestra basicranialis posterior. A somewhat vertical, subparachordal plate is thus formed which is perforated by a large opening, traversed by the arteria carotis interna. That artery, after traversing this opening, passes through the fenestra hypo-

physeos, posterior to the hypophysis, sends a cross-commissural branch to its fellow of the opposite side, another branch, the arteria ophthalmica interna, outward dorso-anterior to the polar cartilage, and is then itself distributed to the brain. It passes mesial to the polar cartilage of its side, but lateral and dorsal to the processus infrapolaris. If this latter process were to become the only connection between the polar cartilage and the basal plate, the artery would pass lateral to the polar cartilage, and this is apparently what actually takes place in the Mammalia, as will be explained later.

Comparing these conditions in the chick and duck with those in embryos of the Teleostei and Holostei, it is at once evident that the cartilago acrochordalis of the former must be the homologue of that cartilaginous prootic bridge of the latter which forms the beginning of the definitive prootic bridge. The relations of these two cartilages to the other skeletal elements, and to the brain, are too strictly similar to leave any reasonable doubt as to this, the differing relations of the cartilages to the notochord evidently being related to the early development of the cartilage in the chick and duck and its late development in the Teleostei and Holostei. The space which, in the Teleostei and Holostei, lies between this bridge and the otic portion of the basal plate must then be the homologue of the fenestra basicranialis posterior of the chick and duck, as has already been stated, and the side walls of this fenestra the homologues of the basiotic cartilages; these latter cartilages being prolonged ventrally, in fishes, by the ventral processes of the prootics, and, in the chick and duck, by the infrapolar processes. These latter processes, together with the polar cartilages, are then the so-called anterior prolongations of the parachordals of Swinnerton's and Gaupp's descriptions of *Gasterosteus* and *Salmo*, there apparently developed in continuity with the basiotic cartilages, as they are said to be in certain of the Aves. The so-called fenestra basicranialis posterior, or fenestra interparachordalis, of Gaupp's and Swinnerton's descriptions of fishes is then the homologue of the fenestra hypophyseos of the chick and duck and not of the fenestra basicranialis posterior, and the fenes-

tra hypophyseos of embryos of fishes has been suppressed in advanced embryos of the chick and duck.

In Talpa, Noordenbos ('05) finds the parachordals of opposite sides, when first developed, united with each other, ventral to the notochord, and not extending to its tip. The tip of the notochord reaches, at this stage, to the hypophysis, and is said to represent, in a certain sense, the morphological anterior end, or anterior pole, of the embryo, the hypophysis being an organ at that pole. In slightly older embryos the notochord is somewhat withdrawn from the hypophysis, and its tip then doubtless lies posterior to the infundibulum. The parachordal plate has at the same time grown rostralward, and, turning upward at its anterior end, now surrounds the notochord, which traverses it from its dorsal to its ventral surface and extends anteriorly beyond it.

The trabeculae first appear as a single median plate between the nasal sacs and extending posteriorly to the recessus preopticus. In the space between the trabecular and parachordal plates, ventral to the hypophysis and at a slightly lower level than the parachordal plate, two pairs of little cartilages, the insulae polares, later appear, and soon fuse to form a polar plate which is at first perforated by a median fenestra hypophyseos, which soon becomes closed by growth of the bounding cartilage. This polar plate fuses, soon after its formation, with the trabecular plate, and in the line of fusion a slight transverse furrow is formed which lodges the chiasma opticum. The hind edge of this furrow is slightly raised, and forms the tuberculum sellae, which thus lies on the anterior end of the polar plate and not, as in the chick and duck, on the hind ends of the trabeculae. No cartilago acrochordalis has yet been formed, and the polar plate accordingly cannot fuse with the basal plate along the line of fusion of that cartilage with the cartilagine basioticae, as it does in the chick and duck. Accordingly, a direct fusion of the polar plate with the basal plate does not take place, and connection with the latter plate is acquired through the intermediation of a delicate Y-shaped mass of cartilage, the arms of which fuse with the projecting anterior ends of the

parachordal plate. This Y-shaped cartilage, probably together with the posterior pair of insulae polares, thus corresponds to the infrapolar processes of the chick and duck, and the internal carotid arteries run upward lateral to them, as they do in the chick and duck and as explained just above.

The Y-shaped cartilage of *Talpa*, by its fusion with the anterior end of the parachordal plate, encloses a circular opening which Noordenbos calls the fenestra basicranialis posterior. The anterior end of the notochord lies directly above this fenestra, which it would not do were the fenestra the homologue of the similarly named fenestra in the chick and duck. Furthermore, Noordenbos says ('05, p. 385) that the hypophysis lies in a slight fossa, bounded anteriorly by the tuberculum sellae (a ridge formed, as above stated, on the anterior end of the polar plate) and posteriorly by the anterior end of the parachordal plate, thus necessarily lying directly above the so-called fenestra basicranialis posterior, instead of, as in the chick and duck, definitely anterior to it. This fenestra of *Talpa* must then be an opening corresponding to some part of Gaupp's fenestra basicranialis posterior of *Salmo*, and apparently to that part of it which he says leads from the middle into the posterior sections of his descriptions of the myodome. The fenestra of *Talpa* is, in any event, not a perforation of the floor of the cavum cerebrale cranii, as it is in the chick and duck, and that perforation, and a cartilago acrochordalis are both wanting in these embryos. The dorsum sellae of these early embryos is then not the homologue of the postclinoid wall of *Amia* and the *Tel-eostei*, nor of the dorsum sellae of Sonies's descriptions of the chick and duck. It is, however, possible that a cartilago acrochordalis may be developed in later stages than those described by Noordenbos, for Voit shows this cartilage in his figures of embryos of the rabbit, there perforated by an opening, the evident homologue of the fenestra basicranialis posterior of Sonies's descriptions of the chick and duck; and Fawcett ('10), in a work I have not been able to consult, is said by Kernan ('16, p. 621) to have found the dorsum sellae separated from the crista transversa in 19-mm. and 21-mm. human embryos, the dorsum sellae

then there representing the cartilago acrochordalis, and the crista transversa representing the anterior end of the parachordal plate.

Sonies calls attention ('07, p. 406) to the unusual position of the fenestra basicranialis posterior in *Talpa*, and suggests that the posterior pair of insulae polares correspond to the cartilago acrochordalis of his own descriptions of the chick and duck. Terry ('17) says that this fenestra lies, in embryos of the cat, between the anterior end of the parachordal plate and the cartilago polaris (hypophyseal cartilage), thus agreeing with Noordenbos in his identification of it, and he says that it lies "not within the basal (parachordal) plate, but anterior to it, as Noordenbos insists." In his figure of a median vertical section of a 12-mm. embryo (l. c., fig. 17) he, however, shows it lying definitely beneath the turned up anterior end of the parachordal plate, in exactly the position I have assigned to it.

Polar cartilages, lying between the trabeculae and parachordals, have thus been identified in *Acanthias* and *Lepisosteus* among fishes, and in several of the *Sauropsida* and *Mammalia*, and it is probable that they form an integral element of the cranium in all of the *Gnathostomata*, though probably not always developed as wholly independent cartilages. The two cartilages embrace the ectodermal stalk of the hypophysis, the opening between them thus being the fenestra hypophyseos properly so-called, but this fenestra is continued both anteriorly and posteriorly, at certain stages of development, in most of the *Gnathostomata*. The anterior prolongation of it lies between the hind ends of the trabeculae, and although it is generally considered to persist, in the *Teleostei* and *Holostei*, as part of the fenestra ventralis myodomus of the adult, it is probable that it becomes largely, if not entirely, suppressed by fusion of the trabeculae. The posterior prolongation of it lies, in fishes, between the ventral edges of the ventral processes of the prootics, and, in the *Aves* and *Mammalia*, between the corresponding edges of the infrapolar processes. These processes must then be homologous structures, and if the ventral processes of the prootics of fishes are ventrolateral processes, as I conclude,

the intrapolar processes, and hence also the polar cartilages, must be hypochordal and not parachordal structures. This, then, is in accord with, but an extension of, Terry's conclusion ('17, pp. 344 and 396) that there seems no doubt of the presence of a cartilaginous hypochordal layer in the occipital region of mammals generally, and that the basal plate of the occipital region falls into the category of arch structures, not centra.

The notochord and trabeculae may now be considered. Swinerton says that the notochord in *Gasterosteus* undergoes no actual reduction from the earliest to the latest stages examined by him, its relatively less extensive anterior prolongation in older embryos being wholly due to an anterior prolongation of the parachordal cartilages. Froriep, however, says ('02 a, '02 b) that in *Torpedo* there is an actual disintegration of the anterior portion of the notochord. According to him, in early embryos of that fish, the notochord is separated into two definite regions, one of which he considers to be spinal and the other prespinal. The spinal region is said to begin at the dorsorostral corner of the first visceral pouch, this point coinciding with that in which the dorsal wall of the foregut, in early embryos, bends abruptly ventrally in an obtuse angle, the notochord there also bending ventrally at the same angle. Posterior to this point the notochord develops a cuticular sheath immediately after its constriction from the dorsal wall of the foregut, and is persistent throughout the life of the individual. Anterior to this point, and hence in the prespinal region, the notochord presents two different conditions, one related to the region in which the mandibular head cavities develop and the other to that in which the premandibular cavities develop. In the mandibular region a chorda entoblast is said to be constricted from the dorsal wall of the foregut exactly as in the spinal region, but it does not undergo further differentiation and later entirely disintegrates. In the premandibular region, according to Froriep ('92 b, p. 55):

Kommt es nicht einmal zur Bildung einer primitiven Chordaanlage, sondern deren Bildungsmaterial sowohl wie dasjenige des Mesoblasts bleibt ungesondert in der Wand des Vorderdarms enthalten.

Diese indifferente Urdarmmasse schnürt sich zu Ende des Stadium F von den Gebilden der Mandibularregion vollständig ab und stellt nun die Anlage der Prämandibularen Kopfhöhle Balfour's oder das I. Somit van Wijhe's dar.

The protovertebrae are said by Frioriep to extend the full length of the persisting notochord, and not to extend beyond that point; the whole animal being, at this stage, vertebral column. The prespinal, or head region is said to contain the matrix in which all the visceral arches and the mandibular and premandibular head cavities are developed.

Katherine M. Parker, in the latest work I know of relating to this subject, also finds, in the Marsupialia, the notochordal tissue extending anteriorly beyond the end of the persisting notochord, for she says ('17, p. 24):

The primitive relation of the tip of the notochord is one of continuity with the protochordal plate, and in *Perameles* continuity is retained between the chorda and the derivatives of the protochordal plate (prechordal plate and Seesel's pocket). As a secondary condition, continuity may be established between the chorda and the hypophysis.

His, in a much earlier work, also came to a similar conclusion, for he says ('92, p. 348) that the notochord, in all early vertebrate embryos, ends anteriorly in a tapering point which lies immediately posterior to a transverse basal ridge (Basilarleiste) of the brain which lies at the extreme anterior end of the ventral surface of the neural tube. This basal ridge is in contact, either with the dorsal end of Seesel's pocket or with a strip of entoderm (Entodermstreife) which replaces that pocket, and His shows the tip of the notochord wedged in between his basal ridge and Seesel's pocket in two different figures, one said to be a general vertebrate schema and the other to show an actual median sagittal section of the head of an embryo of *Pristiurus* 26-mm. in length. Seesel's pocket lies at the dorsal edge of the oral plate, and is said to be not only topographically, but also genetically, an anterior continuation of the notochord (*l. c.*, p. 350), the notochordal tissue thus extending to the level of the anterior end of the ventral surface of the neural tube. This primitive topographical relation of these four structures, the basal ridge,

the tip of the notochord, Seesel's pocket, and the dorsal edge of the oral plate, is said to be subject to marked changes in later stages and in different vertebrates, Seesel's pocket shifting either anteriorly or ventrally (*rachenwärts*) relatively to the basal ridge. In the latter case an ectodermal fold is formed between it and the basal ridge, and becomes the hypophysial invagination (Rathke's pocket), which extends posteriorly beyond the basal ridge, forcing the tip of the notochord away from the ventral surface of the brain, and even forcing it upward into the *plica encephali ventralis*. The relations of the brain to the notochord, in the adult vertebrate, are accordingly said by His not always to be the primitive ones, and he (*l.c.*, p. 358) considers only those parts of the brain of the adult to be pre-chordal which lie anterior to the basal ridge, and which therefore formed primarily a part of the anterior surface of the neural tube. Those parts are said by him to be the regions of the recessus infundibuli, the chiasma opticum, the recessus opticus, the lamina terminalis, and the olfactory lobes. The saccus vasculosus lies posterior to the basal ridge and belongs morphologically, as well as actually, to the ventral surface of the brain, the line between the morphologically ventral and anterior surfaces of the brain thus lying between the saccus and the recessus infundibuli.

There is thus reason to believe that the notochord extended primarily to the level of the anterior end of the primitive gut, and that, accordingly, the epichordal and hypochordal bands of skeletogenous material, developed in relation to it, had a similar extent. The polar and trabecular cartilages must then be developed from some part of these anterior extensions of these bands, and the polar cartilages quite certainly, as already stated, from the hypochordal bands alone. The trabeculae, in crania of the platybasic type, would seem to be developed from both these bands of tissue. In crania of the tropibasic type the two bands seem to have been forced apart, by pressure of the eyeballs, the epichordal bands lying at the top of the interorbital septum and the hypochordal bands at the bottom of that septum.

It is furthermore to be noted that the trabeculae do not lie

along the ventral surface of the brain, as that surface is defined by His; since as, when first formed, their hind ends apparently always lie anterior to the recessus infundibuli, they must themselves lie either definitely on the anterior surface of the brain or along the lateral surface of its extreme anterior end. In the latter case they would actually have, to the neural tube, the relations of dorsal vertebral arches. It does not, however, necessarily follow that they are such arches, for their relations to the brain may be wholly due to a cranial flexure so sharp and pronounced that it has turned the anterior surface of the neural tube downward upon cartilages which primarily lay either in the line of the axis of the body, or projected ventrally beneath it.

SUMMARY

A functional myodome is found only in fishes, and even among them it is limited, in those I have examined, to *Amia* and the non-siluroid Teleostei.

The myodome is always separated from the cavum cerebrale cranii by membrane (*dura mater*), cartilage, or bone, and the separating wall is in part spinal and in part prespinal in position. A depression in the prespinal portion lodges the hypophysis or both the hypophysis and saccus vasculosus, and this part of the wall never undergoes either chondrification or ossification, a more or less developed pituitary sac always projecting into the myodome.

The myodome is found in its most complete form in the Teleostei, and there consists of dorsal and ventral compartments which are usually separated from each other only by membrane, but that membrane, the horizontal myodomic membrane, is capable of either chondrification or ossification. The dorsal compartment lodges the hind ends of the *musculi recti externi* and is always traversed by a cross-commissural venous vessel formed by the pituitary veins. The ventral compartment lodges the hind ends of the *musculi recti interni* and is traversed by the internal carotid and efferent pseudobranchial arteries and the palatine branches of the *facialis* nerves.

The parasphenoid forms the floor of the ventral compartment of the myodome, and whenever the horizontal myodomic membrane undergoes ossification, the bone so formed forms part of the parasphenoid. This bone is thus certainly, in some fishes, in part of axial origin, and not simply a dermal bone which has gradually sunk inward to its actual position.

In the Siluridae (*Amiurus*) there is apparently a much reduced, but non-functional, dorsal myodomic compartment, but no ventral compartment, that portion of the parasphenoid which lies in the prootic region being developed in what corresponds to the horizontal myodomic membrane of others of the Teleostei.

In *Amia* the myodome corresponds to the dorsal compartment only of the teleostean myodome, and a strictly similar, but non-functional myodomic cavity is found in *Lepidosteus* and *Polypterus*. The ventral compartment of the teleostean myodome is represented, in each of these three fishes, by a canal, on either side of the head, which is traversed by the internal carotid artery, and which corresponds to the *canalis parabasalis* of Gaupp's descriptions of higher vertebrates.

The myodomic cavity is limited, in the Holostei and Crossopterygii, to the prootic region, and is there in part subspinal and in part prespinal and subpituitary in position. In the non-siluroid Teleostei examined, the dorsal compartment of the myodome is always more or less prolonged posteriorly into the basioccipital region and the ventral compartment frequently so prolonged.

The posterior part of the basioccipital portion of the myodome lies between ventrolateral vertebral processes which are quite certainly the homologues of the haemal arches of the tail. In *Hyodon* this part of the myodome is an open groove and lodges the anterior portion of the median dorsal aorta. In the Cyprinidae part of this groove has become enclosed to form a short canal which is traversed by the median dorsal aorta, the enclosing bone forming the pharyngeal process.

The conditions in these fishes thus lead inevitably to the assumption that the entire dorsal myodomic cavity is a sub-vertebral canal similar to the haemal canal in the tail, and that

the dorsal aorta has been excluded from it because of the formation of a circulus cephalicus. What the primary relations of the hypophysis and pituitary veins to this preexisting canal were is problematical, but they became lodged in its anterior portion and so gave rise to the conditions actually found in *Lepidosteus* and *Polypterus*. The muscoli recti externi then secondarily invaded this space by traversing the foramina for the pituitary veins, the other rectus muscles retaining their insertions on the external surface of the preclinoid wall, and so gave rise to the conditions found in *Amia*. The conditions in the non-siluroid Teleostei then arose as a result of the resorption of the cartilage which, in *Amia*, forms the preclinoid wall, the pedicel of the alisphenoid, and those ventral portions of the basicapsular commissures which form the lateral walls of the subpituitary portion of the myodome. Because of the resorption of the preclinoid wall, and its replacement by membrane, the muscoli recti interni, which in *Amia* have their points of insertion on either lateral edge of that wall, have first sought firmer attachment on the dorsal surface of the parasphenoid, and have later pushed posteriorly in the open ends of the persisting portions of the canales parabasales. The fusion of these two canals with each other has formed a ventral myodomic compartment which, in early embryos, is separated from the dorsal and primary compartment by membrane only; but this membrane may undergo either partial chondrification (*Hyodon*) or ossification (*Gasterosteus*), the bone, in the latter case, forming a transverse and inclined ridge on the dorsal surface of the parasphenoid. The membranes resulting from the resorption of the preclinoid wall were then pressed together in the median line by the recti interni, and form a median vertical myodomic membrane which encloses the internal carotid arteries in a membranous canal, the homologue of the cartilaginous canals of *Amia*. The efferent pseudobranchial arteries, pressed downward by the recti interni, lost their connections with the internal carotids and acquired a cross-commissural connection with each other. The membrane resulting from the resorption of the anterior portions of the basicapsular commissures of either side ossified as part of the

ascending process of the parasphenoid, and the tissues resulting from the resorption of the pedicel of the alisphenoid ossified, in certain fishes (*Cottus*, *Gasterosteus*), to form an anterior portion of that process.

The myodomic cavities of the Holostei and Teleostei are represented in the Selachii either by canals in the basis cranii which are traversed by the pituitary veins and the internal carotid and efferent pseudobranchial arteries or by a posterior and deeper portion of the large pituitary fossa of the chondrocranium which is shut off from the cavum cerebrale cranii by the dura mater, and is traversed by the pituitary veins and the internal carotid arteries.

In embryos of *Ceratodus* there is a subpituitary space, traversed by the pituitary veins, which corresponds to the dorsal compartment of the teleostean myodome, and the internal carotid canals of *Amia* have been added to it. This fusion of these canals with the dorsal myodomic cavity is due, either to the resorption of the cartilage that separates them in *Amia* or to a shifting posteriorly of both the hypophysis and the internal carotids from a position between the hind ends of the trabeculae to one between the so-called anterior prolongations of the parachordals.

In the Amphibia the basis cranii apparently corresponds to the roof, and not to the floor, of the dorsal myodomic cavity of *Amia* and the Teleostei. The fenestra hypophyseos of these animals is then the homologue of the pituitary opening of the brain case of fishes.

The Reptilia and Mammalia have a dorsal myodomic cavity similar to that in *Ceratodus*. In man it is represented in the cavernous and intercavernous sinuses, and the venous vessels that traverse the sinuses are the homologues of the pituitary veins of fishes.

The cartilago acrochordalis of Sonies' and Noordenbos' descriptions of birds and mammals, respectively, is the homologue of the cartilaginous prootic bridge of embryos of fishes. The open space between this cartilage, or bridge, and the anterior end of the parachordal plate is the fenestra basicranialis poste-

rior proper. This fenestra is a perforation of the roof of the myodomic cavity, and hence is not the homologue of the so-called fenestra basiscranialis posterior of embryos of fishes, which is a perforation of the floor of that cavity. This latter fenestra of embryos of fishes is the homologue of the fenestra hypophyseos of birds and mammals, the so-called anterior prolongations of the parachordals of fishes being the homologues of the polar cartilages of birds and mammals.

In certain of the Selachii there is an acustico-trigemino-facialis recess, and there may be certain canals in the cranial wall traversed by the vena jugularis and the external carotid artery.

In *Amia* the trigemino-facialis portion of this recess has fused with the canals for the vena jugularis and the external carotid artery to form a trigemino-facialis chamber; this chamber has become continuous with the myodome, and the large chamber so formed has been prolonged anteriorly by a space between the pedicel of the alisphenoid and the primitive side wall of the neurocranium. The foramina for the pituitary vein and the oculomotor and trochlear nerves open into this anterior prolongation of the chamber, and through its orbital opening into the orbit. The vena jugularis traverses this opening to enter and traverse the trigemino-facialis chamber; the musculus rectus externus traverses it to enter the myodome, and the nervus profundus traverses it to join the ganglion, or root of the nervus trigeminus. The nervus trigeminus and the external carotid artery issue from the trigemino-facialis chamber posterior to the pedicel of the alisphenoid and run forward lateral to it.

In the non-siluroid Teleostei the trigemino-facialis chamber is not continuous with the myodome, and it has been separated by a wall of bone into ganglionaris and jugularis parts which correspond, respectively, to the trigemino-facialis recess and the jugular and external carotid canals of the Selachii. The pedicel of the alisphenoid is incomplete or wholly wanting, but it may be replaced by an anterior prolongation of the ascending process of the parasphenoid. In the latter case the nerves, arteries, veins, and muscles all have the same relations to this process that they have to the pedicel of the alisphenoid of *Amia*. The

lateral wall of the pars jugularis of the trigemino-facialis chamber is always less extensive than in *Amia* and may be wholly wanting.

In *Ceratodus* there is a trigemino-facialis chamber similar to that in *Amia*, and there is a bar of cartilage which corresponds to the pedicel of the alisphenoid of that fish.

In the Amphibia there is a trigemino-facialis recess, and the pars ascendens of the quadrate forms the lateral wall of a space corresponding to the pars jugularis of the chamber of the Teleostei. The ascending process of the palatoquadrate is the homologue of the pedicel of the alisphenoid of fishes.

In the Reptilia there apparently is no trigemino-facialis recess, the lateral wall of the neurocranium being the primitive cranial wall. The pars ascendens of the quadrate forms the lateral wall of a trigemino-facialis chamber. The antipterygoid (columnella) is the homologue of the pedicel of the alisphenoid of fishes, and the processus basipterygoideus the homologue of the floor of the orbital opening of the myodome of *Amia*.

In the Mammalia there is a trigemino-facialis recess formed by the cava epiptericum and supracochleare. The ala temporalis is peculiar to mammals; it is a bar of cartilage formed between the nervi maxillaris and mandibularis trigemini as they issue from the trigemino-facialis recess, the processus alaris corresponding to some part of the side wall of the prespinal portion of the myodome of *Amia*. The ala temporalis has been prolonged anteriorly so as to enclose a space anterior to the trigemino-facialis recess, and the foramina for the pituitary vein (sinus cavernosus) and the nervi oculomotorius, trochlearis and profundus (first branch of trigeminus) open into this space and from it into the orbit. The cavum tympanicum is the pars jugularis of the trigemino-facialis chamber, and the processus pterygoideus, the malleus, incus, and stapes, and probably also the annulus tympanicus, are quite certainly portions of the lateral wall of that part of the chamber. A diverticulum of the spiracular canal, or an independent evagination of the pharynx, has expanded into this part of the chamber and so formed the middle ear. The chorda tympani must then correspond to that communicating branch from the nervus facialis to the nervus

trigeminus which, in fishes, traverses the trigemino-facialis chamber, and hence must be a prespiracular nerve.

The internal carotid artery enters the cranial cavity, in most vertebrates, by passing upward mesial to the related trabecula, or mesial to the posterior prolongation of the trabecula formed by the polar cartilage, but in *Amiurus* it enters the cranial cavity through the foramen opticum, and hence would there seem to pass lateral and then dorsal to the trabecula. In embryos of the Mammalia ditremata this artery is said to also pass upward lateral to the trabecula, but it is probable that it here simply passes lateral to the infrapolar process of the polar cartilage, the latter cartilage not itself fusing directly with the parachordal plate, and its direct relations to the artery thus being obscured.

Palais de Carnolès, Menton, France,
May 1, 1918

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Figs. 1 to 12. Semidiagrammatic figures of transverse sections through a 51-mm. specimen of *Hyodon tergisus*, showing the myodomic cavities and adjacent regions, and selected at intervals between the hind end of the interorbital septum and the hind end of the basiocephal.

Figs. 13 to 18. Similar sections of a 20-mm. specimen of *Cottus scorpius*.

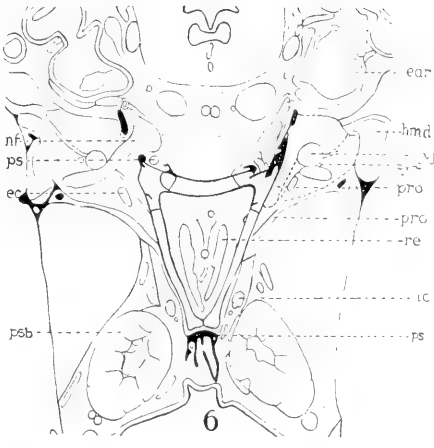
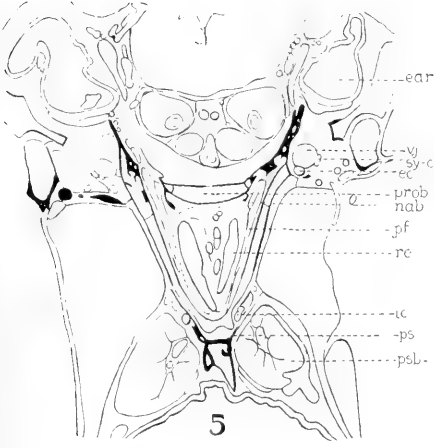
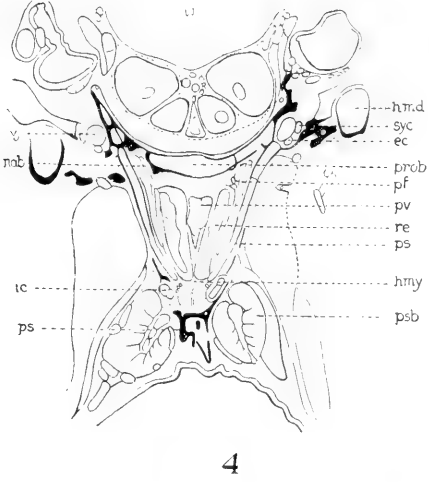
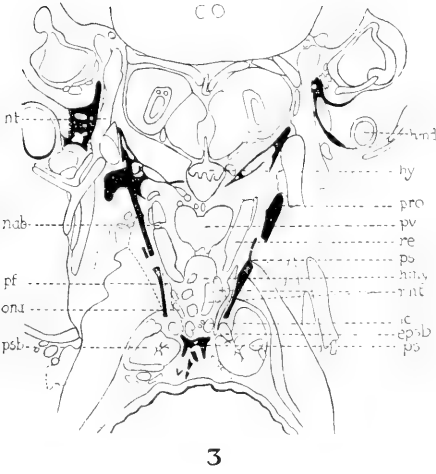
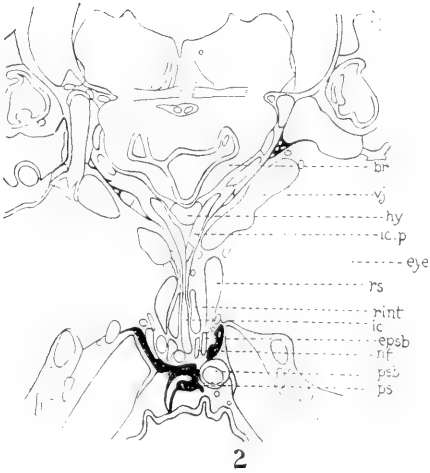
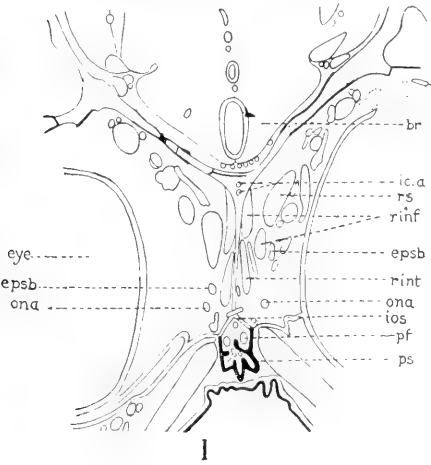
Fig. 19. Similar sections of a 31-mm. specimen of *Clinocottus analis*.

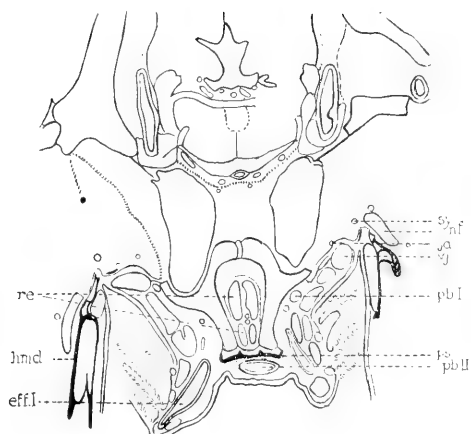
Figs. 20 to 23. Similar sections of a 115-mm. specimen of *Syngnathus acus*.

Figs. 24 to 29. Similar sections of a 57-mm. specimen of *Catostomus occidentalis*.

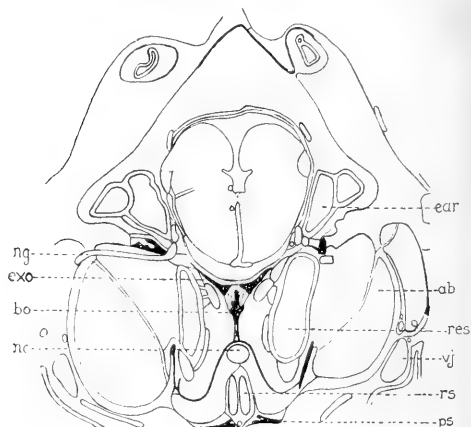
ABBREVIATIONS

<i>ab</i> , air-bladder	<i>ng</i> , nervus glossopharyngeus
<i>as</i> , aortal support	<i>nocm</i> , nervus oculomotorius
<i>ba</i> , basiocephal	<i>nl</i> , nervus trigeminus
<i>br</i> , brain	<i>ona</i> , orbitonasal artery
<i>dlp</i> , dorsolateral vertebral process	<i>pb. I</i> , pharyngobranchial of first branchial arch
<i>ear</i> , parts of membranous ear	<i>pb. II</i> , pharyngobranchial of second branchial arch
<i>ec</i> , external carotid artery	<i>pf</i> , ramus palatinus facialis
<i>eff. I</i> , efferent artery of first branchial arch	<i>php</i> , pharyngeal process
<i>epsb</i> , efferent pseudobranchial artery	<i>pro</i> , prootic
<i>exo</i> , exoccipital	<i>prob</i> , prootic bridge
<i>hmd</i> , hyomandibula	<i>ps</i> , parasphenoid
<i>hmy</i> , horizontal myodomic membrane	<i>psb</i> , pseudobranch
<i>hy</i> , hypophysis	<i>pv</i> , pituitary vein
<i>ic</i> , internal carotid artery	<i>re</i> , musculus rectus externus
<i>ic. a</i> , anterior division of internal carotid artery	<i>res</i> , recessus sacculus
<i>ic. p</i> , posterior division of internal carotid artery	<i>rinf</i> , musculus rectus inferior
<i>ios</i> , interorbital septum	<i>rint</i> , musculus rectus internus
<i>ja</i> , Jacobson's anastomosis	<i>rs</i> , musculus rectus superior
<i>l</i> , ligament	<i>sv</i> , saccus vasculosus
<i>lda</i> , lateral dorsal aorta	<i>syc</i> and <i>sy-c</i> , sympathetic nerve and communicating branch from N. trigeminus to N. facialis
<i>mda</i> , median dorsal aorta	<i>vj</i> , vena jugularis
<i>nab</i> , nervus abducens	<i>vlp</i> , ventro-lateral vertebral process
<i>nc</i> , notochord or notochordal space	
<i>nf</i> , nervus facialis	

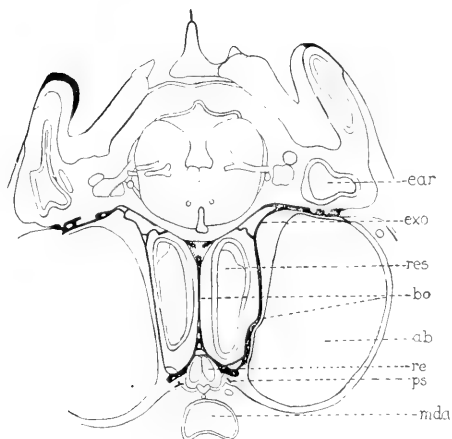




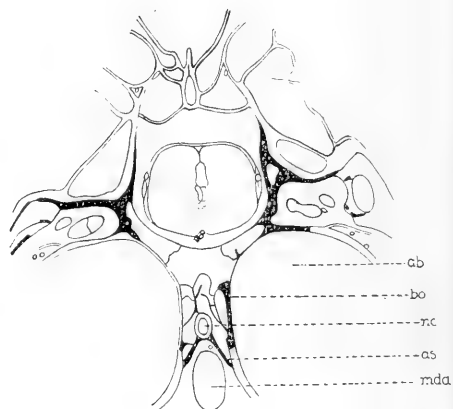
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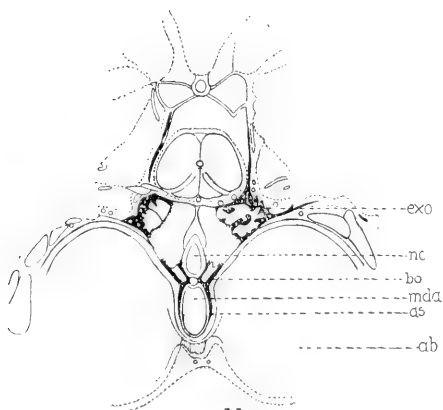
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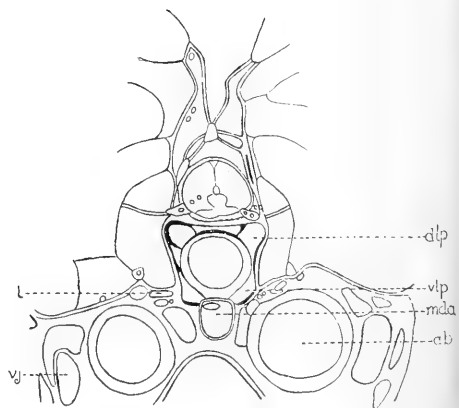
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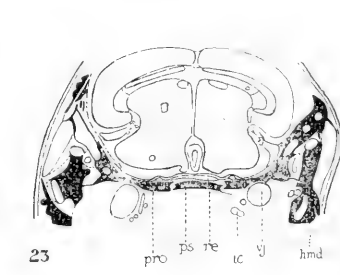
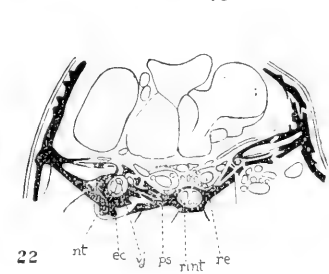
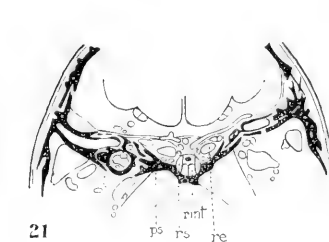
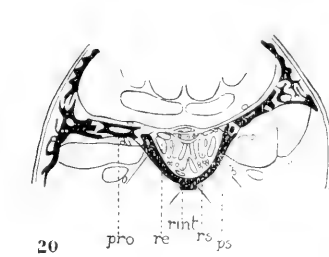
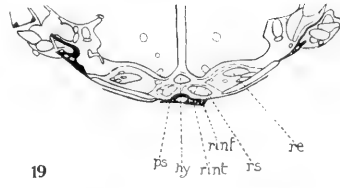
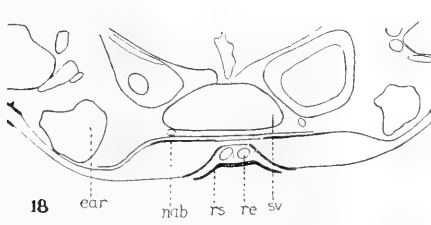
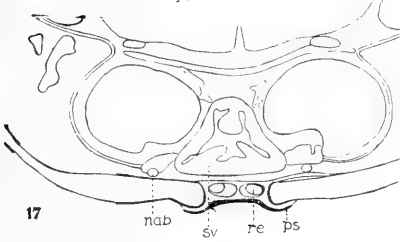
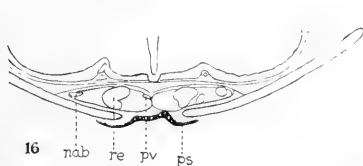
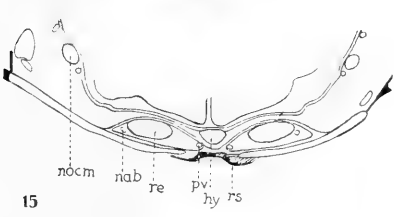
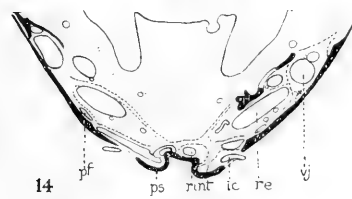
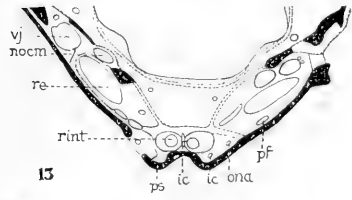
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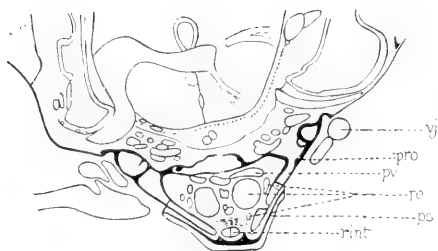


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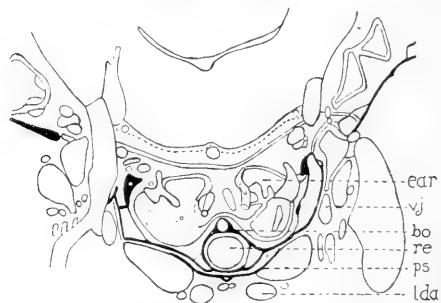


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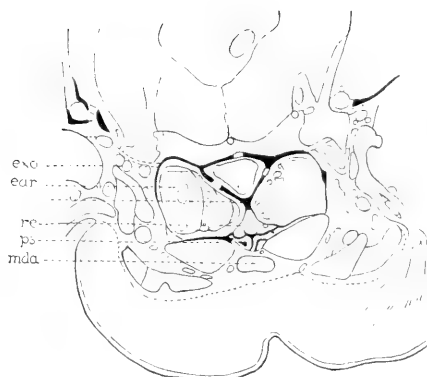




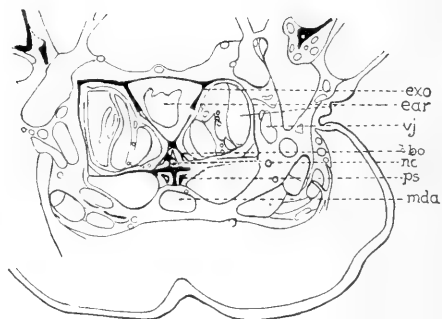
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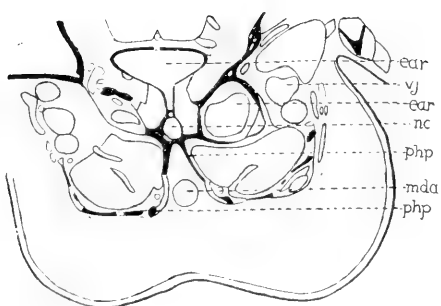
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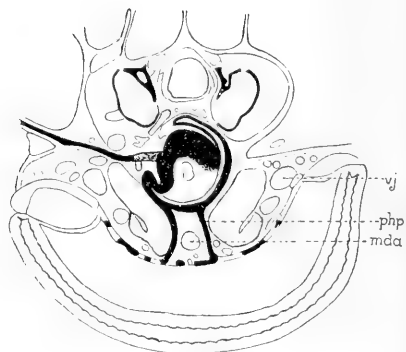
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ON THE NATURE, OCCURRENCE, AND IDENTITY OF THE PLASMA CELLS OF HOFBAUER

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The history of these cells illustrates very well how a re-discovery, when accompanied by a fuller description, succeeds in domiciling itself in anatomical literature as an original discovery. As we shall presently see, Hofbauer ('05) was impressed especially by a conspicuous phase in the life history of a particular cell. He noted its reaction in the fresh state, to certain stains, described it more fully, and speculated with some freedom on its functional rôle; but he did not discover this cell, as he supposed, in 1903. Although Hofbauer refers to his address given in 1903 in his book published in 1905, he does not refer to or list the paper based on this address, published in 1903, in the title of which he refers to these cells as 'hitherto unknown' and as 'constantly occurring.' His failure, in 1905, to recognize earlier workers was, I presume, an oversight, which apparently led Essick ('15) and others to assume that "Hofbauer first called attention to specific round cells appearing in the human placenta toward the end of the fourth week of pregnancy."

The type of cells which in recent years has been designated with Hofbauer's name was known previously especially as Wanderzelle and had been represented by various investigators. Minot ('12), in a footnote, refers to the latter fact and rightly adds: "It has long been known that strikingly large free cells appear in the mesenchyme of the chorion. They are pictured in my Human Embryology." Reference to the illustration in this work shows a large, rather granular cell, with a somewhat eccentrically placed, vesicular nucleus, but without vacuoles. Moreover, previous to the publication of the Embryology,

Minot ('89) not only spoke of large, granular, wandering cells in the stroma of the chorion, but also represented them. From Minot's familiarity with the work of Langhans ('77) and of Kastschenko ('85), it does not seem unlikely that, among others, he had these investigators particularly in mind when he referred to the earlier descriptions.

In the absence of a more discriminating term for these erratic and ephemeral elements, the original designation of wandering cell would seem far preferable to the designation lipid interstitial cells, used by certain Italian writers. The former is a non-committal term, and, although too inclusive, is for this reason no more objectionable than the expression giant-cell. Although these cells may not—indeed, probably do not—wander in the sense of the amoeba or the leucocyte, they nevertheless may change their location decidedly. The qualification, intersitital, is objectionable for the very reason for which it was chosen—the alleged analogy to the interstitial cells of testis and ovary, and since they may contain lipid substances merely because they are degenerate, the adjective lipid is equally objectionable. For reasons to appear later, the designation plasma cell, used by certain Italian writers after Hofbauer, would not seem to be justified.

Virchow ('67) stated that isolated cells with clear vesicular spaces in their protoplasm are found in the stroma of the villi in cases of hydatiform degeneration, and identified them with certain other cells, physaliphores—previously described by him. He found these bubble-like cells, as he called them, also in the thymus of the new-born, in cancer, etc., and, according to Virchow, they were not merely vacuolated cells. He seems to have regarded these cells as identical also with the vacuolated syncytial masses, for he stated that Müller described them as occurring in the chorionic epithelium. Since syncytial elements not rarely are found in the stroma, instances of confusion of these two cell types can be found in contemporary literature also.

Langhans ('77), in describing the stroma of the villi, said that it contained "sharply delimited large cells with many granules in the protoplasm. Their form is variable—circular,

spindle and star-shaped." These cells were said to lie mainly near the periphery. However, Langhans, who was interested mainly in other problems, did not represent them nor discuss their probable significance. But Kastschenko ('85) represented them and described them as being about 9μ large, and as corresponding exactly in form and size to the white blood cells of the same embryo. According to Kastschenko, the cytoplasm is reduced in quantity after the first month, so that the nucleus no longer is surrounded by it. The nuclei, also, are said to undergo a change and to appear later as solid structures. The latter observation cannot fail to remind one of pycnosis and of one of its well-known significances. Kastschenko found these cells mainly near the epithelium of the villi and stated that they vary greatly in size, number, and occurrence in the same placenta. The fact that Kastschenko identified the cells found in the mesenchyme of the embryonic villi as leucocytes might seem to indicate that what he saw and described were other than Hofbauer cells. However, his illustrations, especially when considered in connection with those of earlier investigators and those of Minot, leave little doubt that all these investigators saw the same type of cell. Moreover, it is not improbable that Kastschenko was influenced in his interpretation of these cells by the origin and current use of the term *Wanderzelle*. It will be recalled that von Recklinghausen ('63) showed that the leucocyte preeminently belonged in this class of cells, but even at the time that Kastschenko was writing and far later, all cells which were regarded as foreign to the tissue in which they lay still were included in the designation *Wanderzelle*. Reference to the literature of that period will make this fully evident.

The presence of these cells in pathologic 'ova' was noticed repeatedly by Mall ('08), who also designated them as wandering or migrating cells in his earlier protocols. Chaletzky ('91) also saw and described these cells, but perhaps the best description from an earlier date is that given by Kossman ('92), who also referred to the Hofbauer cells as *Wanderzellen*, and gave excellent representations of them. Indeed, from an inspec-

tion of the latter alone there can be no question as to the identity of these Wanderzellen and the Hofbauer cell. In speaking of them, Kossman said:

. . . . Auffallend sind zahlreiche grosse Zellen, die eine sehr wechselnde, oft amöboide, niemals sternförmige Gestalt haben. Die Filarmasse ihres Protoplasma's ist durchaus fein netzartig angeordnet und färbt sich stark in Hämatoxylin. Die Zellen enthalten einen oder mehrere grosse blasenartige Hohlräume, von denen ich leider nicht sicher sagen kann, ob sie Fett führten, da sie mir erst nach Behandlung des Präparats mit Xylol auffielen. Der Kern dieser Zellen enthielt stets Nucleoli. Die Zellen sind also jedenfalls nicht in lebhafter Vermehrung; wahrscheinlich sind es Wanderzellen und da sie auf einem wenig älteren Stadium wieder fehlen, mag ihr Vorkommen in einigem Zusammenhange mit der um diese Zeit beginnenden Vascularisation des Stroma's stehen.

Merttens ('94) found the same cells in abortuses, and, in describing the stroma of the villi of his first case said:

An den Ernährungszotten ist es kernreich, vielfach aufgelockert, mit stern- und spindelförmigen Zellen, in den Maschen jene oben für die normalen ersten Stadien beschriebenen grossen, runden oder polyedriscen Zellen mit körnigem oder auch vacuolärem Protoplasma mit grossem, bläschenförmigem, rundem Kern.

Merttens seems also to have suggested that these cells are swollen stroma cells, but since he made this observation somewhat disconnectedly I am not quite certain of his meaning; yet the mere suggestion is particularly interesting in view of Minot's special emphasis upon the degenerate character of the Hofbauer cells. Marchand ('98) also wrote: "Die durchsichtigen hellen Zellen im Stroma normaler oder pathologischer Zotten sind mir wohlbekannt, sie können denen der Zellschicht sehr ähnlich sein; ich halte sie jedoch für gequollene, rundlich gewordene Bindegewebszellen, da man Übergänge zu solchen findet, ebenso wie in andern Schleimgewebe."

Ulesco-Stranganowa ('96), who also saw these cells, says that if one compare the Langhans cells with round nuclei with these cells scattered about the stroma of the villi, and which have been named Wanderzellen by Kastschenko, one becomes convinced of the identity of these two types of cells. According

to Ulesco-Stranganowa, then, the Hofbauer and Langhans cells are identical. Mall ('15) also called attention to this possibility, for, when speaking of the invasion of the mesoderm of the villi by trophoblast, he called attention to the presence of numerous Hofbauer cells, and added: "It would seem possible that these Hofbauer cells are free trophoblast cells within the mesoderm of the villus, an opinion already expressed in my paper on monsters." Neumann ('97) also noticed these cells and referred to Virchow's opinion regarding them, and von Lenhossek ('02) is credited in 1904 by the reviewer of his paper with having examined a large series of young human embryos, and having suggested that what Kastschenko regarded as Wanderzellen were mesenchyme cells. It should be noted, however, that von Lenhossek apparently came to this conclusion largely because of the absence of blood-forming organs or lymphatic centers in embryos the villi of the chorionic vesicles of which contained these cells. Strangely enough, Kworostansky ('03) also recorded the presence of these cells, and after describing the stroma of the villi wrote:

Zwischen den genannten Bindegewebszellen giebt es in der wolrigen Grundsubstanz Lücken, und am Rande oder im Winkel derselben sitzen freie andere Bindegewebszellen, die sehr gross sind, lappige, runde Form, wabenartiges Protoplasma und gleiche Kerne wie andere Bindegewebszellen haben; ihre Kerne werden auch, hie und da sternförmig getheilt. Da sie stets nur in Gewebslücken gefunden werden, so glaube ich, sie als Lymphgefässendothelien, oder vielleicht als Lymphocyten bezeichnen zu dürfen. Man findet sie in späteren Stadien der Placenta nur sind dann natürlich die zellen nicht mehr gross.

The illustration which accompanies Kworostansky's article, as well as his description, leaves no doubt that the cells seen by him are the same as those which we are considering, although his surmise that they are lymphocytes and that they arise from the endothelium of the lymphatics may, upon first thought, seem rather irreconcilable with such an interpretation.

From these references alone it is evident that Minot's statement, that the so-called Hofbauer cells were repeatedly mentioned in the earlier literature, is well founded. Muggia ('15) states that these cells were described also by Guicciardi ('99),

Clivio ('03), Stoffel ('05),¹ Vecchi ('06), and Pazzi ('04). Indeed, many other names could be added, for surely any one of the many who studied even a small series of chorionic vesicles must have seen some of them in some villi, especially in unrecognized cases of hydatiform degeneration, but since they have been referred to as Hofbauer cells, it is his description that especially interests us. In describing the chorionic villi, Hofbauer ('05) spoke of certain gaps or spaces between the meshes of the mesenchyme of the villi which he thought might belong to the lymphatics or contain tissue fluid. In these spaces he found certain granular, round cells arranged longitudinally. He thought they often were spherical with a diameter of $10.5\ \mu$ to $12.5\ \mu$ but more commonly star-shaped or branched. By means of these branches they come into direct relation with other similar cells or with connective-tissue cells. However, Happe ('06) stated that he could not with certainty find cells united by their processes, as described by Hofbauer, in preparations stained after Hänsen. According to Hofbauer, the cell processes are delicate, and the cells contain one or two nuclei from $4.7\ \mu$ to $5.7\ \mu$ in diameter, oval or circular in form, eccentric in position, with a definite membrane and a dense chromatin network. Mitoses were common, and fragmentation of nuclei and indications of pluripolar mitoses also were seen. Hofbauer emphasized that the most characteristic thing in these cells which he regarded as being specific was the presence of vacuolation in the 'plasma' and the existence of a perinuclear clear zone, which was said to be the result of fusion of 'small light spots.' As the cytoplasm becomes vacuolated the nucleus is said to become pyknotic, which stage is followed by failure to stain and finally by complete disappearance. Hofbauer also noticed the presence of granules and fat droplets, and regarded the life history of the cell as a circumscribed one. He did not find them present in real young villi. They were said to appear at the end of the fourth week, and were more common in young than in old placentae. They reacted to vital stains like plasma cells, and Hof-

¹ A rereading of Stoffel's article shows quite conclusively that he did not describe the plasma cell of Hofbauer.

bauer regarded the vacuoles as having an assimilative and digestive function. A reference to the plates accompanying Hofbauer's monograph, however, shows that vacuolation was not always present, and that the largest of the cells were almost twice the size of the smallest.

In his earlier paper Hofbauer ('03) also said that his preparations taken from material from the fourth to the ninth week of pregnancy, and obtained at operation, showed these cells in all stages of mitotic division. Hofbauer further wondered whether the spaces surrounding these cells are lumina of capillaries, added that the cells discovered by him undoubtedly are found in capillaries, and made some rather unguarded surmises concerning them.

Berlin ('07), in writing on the changes in retained placentae, also spoke of large swollen, hydropic cells which lie in spaces. These cells she regarded as undoubted mesenchyme cells. However, Berlin did not believe that they are degeneration products, although her description certainly would lead one to suppose that they were such. Even when she states that they bear no sign of degeneration, emphasizing that the chromatin network is fine, she speaks of swollen nuclei which have gathered a larger amount of protoplasm about them, phenomena which she regarded as signs of luxurious nutrition. Moreover, Berlin never observed mitoses and never found the nuclei increased in villi containing many of these cells, an observation wholly in harmony with that of others and directly opposed to proliferation.

Grosser ('10), who was plainly aware of the fact that Hofbauer was not the discoverer of these cells, also represented a cell which, however, is non-vacuolated and binucleated, and added that their significance is still unknown.

I have given Hofbauer's description, partly to emphasize the vacuolation, for it was this which also impressed Minto, ('04), who rightfully stated:

We frequently find in the literature mention of wandering cells with vacuolated protoplasm, but they seem not to have been recognized as degenerating cells. . . . The disintegration by vacuolation has, so far as known to me, not been described heretofore, and

consequently may be treated somewhat more fully. Renewed investigation has led me to the conclusion that we have to do with erythrocytes which have gotten into the mesenchyma and, remaining there, have swollen by imbibition and are undergoing degeneration by vacuolization of their protoplasm. . . . We can explain the appearance of these cells by the assumption of imbibition, in which the nucleus has participated. . . . Since I have found similar cells in a considerable number of placentas, I draw the conclusion that they are constant and normal. I regard the interpretation of the pictures unattackable as proof of progressive degeneration.

In association with these remarks, Minot represented a series of cells showing progressive degeneration, beginning with the nucleated red cells and ending with a highly degenerated, but nevertheless nucleated, Hofbauer cell which apparently is in process of disintegration. These cells were seen by Minot especially in a human embryo of 15-mm. length, from the Mall collection.

As shown in the references to the literature above, it is not quite correct to say that the degenerate character of vacuolation has not before been recognized, for the surmises that Hofbauer cells contain fat granules may, and that they are swollen mesenchyme cells must, carry this implication. Moreover, those familiar with the effects of inanition know that investigators of this subject long ago called attention to vacuolation as one of the evidences of degeneration although, certainly, no one contends that it always is such.

Instead of regarding these cells as degeneration products, certain Italian writers (notably Acconci '14) regard cells which they found, especially in the first half of pregnancy, as morphologically and functionally comparable to the interstitial cells of the ovary and testis. Acconci believed that certain cells which he and other Italian writers after him designated lipid-interstitial cells, probably produce a special internal secretion. He, like Hofbauer, found these cells to contain lipid granules, and regarded them also as equivalent to certain cells "described by Ciaccio in various parts of the organism, or by Brugnattelli in the interstitial tissue of the mammary gland." Acconci further emphasized certain similarities between the syncytium

and the interstitial cells, both of which he conceived as exercising a protective rôle. Muggia ('15), too, instead of regarding the lipoid interstitial cells of Acconci as degenerate, emphasized his belief that they are particularly resistant to degeneration, being found perfectly preserved in the midst of detritus. Since the young connective-tissue cell loses, or rather retracts, its processes as it becomes converted into a Hofbauer cell, it need not surprise us that the latter survives the former. Retraction of the processes contributes to the apparent increase of cytoplasm of the rounded swollen cell and also is involved in the formation of the spaces in which these cells usually lie. Muggia, who considered the cells found by him in great numbers in a case of partial hydatiform degeneration, as identical with those described by Acconci, gave a fine detailed description absolutely typical of the cells described in greatest detail by Hofbauer. Moreover, the excellent illustrations which accompany Muggia's article leave no doubt as to the identity of the cells or of their degenerate character. Muggia stated that these cells in normal villi increase until the end of the fifth month, when, according to Savare, they are most numerous. Muggia further found numerous cells very similar to the interstitial cells of Acconci, or "the plasma-like cells of Hofbauer," which he says are regarded by some as early stages of interstitial cells and by others as mast cells, although he regarded them as partially differentiated interstitial cells.

Until I had seen sections of the chorion of embryo no. 1531, I was largely at a loss to know why Hofbauer cells so frequently were described as lying in gaps or spaces in the mesenchyme. However, in this specimen cross-sections of a number of villi showed splendid examples of this condition, which alone made the cells very conspicuous. The cells often were very numerous, in fact more numerous than the mesenchyme cells which remained, although some well-preserved villi contained no Hofbauer cells whatever. Some of the younger specimens also contained none. This was true of a chorionic vesicle with an embryo 1 mm. in length. They were found most commonly in the villi, but not infrequently some of them lay in areas of

the chorionic membrane which had undergone degeneration. They were not so common here, but sometimes were exceedingly numerous in small areas. They were found in the amnion also, in the umbilical cord, and in the tentorium cerebelli, and as isolated specimens in embryonic mesenchyme elsewhere. As emphasized by other investigators there seemed to be nothing particularly characteristic about their distribution except that they were more common in places where the mesenchyme was degenerating. Sometimes a considerable number were contained in one villus and none in an adjacent one. As many as twelve might lie in one field and none in the next. Very rarely was there a solid mass of them, but usually they were scattered about at random, although groups also were seen. The better-preserved cells were small, the poorer-preserved larger, the size varying from $8.5\ \mu$ to $30\ \mu$. The smaller cells usually were quite circular in outline, stained evenly and possessed a non-granular cytoplasm with a nucleus quite centrally located. Binucleate cells, as described by Grosser, were not uncommon, and multinucleated cells—fusion products—also were found. The nuclei of the latter frequently were more unequal in size, and usually also more oval in outline, than the single nucleus of the typical Hofbauer cell. Measurements of the larger cells made with a micrometer caliper, gave the following results which are considerably above those given by Hofbauer, whose estimation of $10.5\ \mu$ to $12.5\ \mu$ applies to the average-sized cell.

Size of the larger Hofbauer cells in micra

25.5 \times 20.4

30.4 \times 27.5

18.0 \times 12.0

21.5 \times 25.5

18.0 \times 14.0

However, the size of the cells varied from specimen to specimen of chorionic vesicle, but not nearly so much as their state of preservation. This no doubt, partly is due to the varying state of preservation of the villi themselves.

In outline they varied from irregular to circular, as stated by Hofbauer, and as represented by Minot ('11) in his series showing progressive degeneration. Although it was easy to distinguish the vacuolated Hofbauer cell from the well-preserved mesenchyme cell with cylindrical nucleus and many processes, specimens which represent transition forms as stated by Marchand, were quite common. The latter generally were oval or slightly irregularly formed cells with a number of short processes, which latter, as well as the character of the nuclei and the form of the cell itself, certainly suggested a mesenchymal origin. They were also most numerous in villi the stroma of which had become vacuolated or fenestrated. Here the reciprocal numerical relationship between the Hofbauer and the mesenchyme cells often was especially evident. In certain areas in which almost no mesenchyme cells remained intact, numerous Hofbauer cells occurred in all stages of degeneration. In other portions of the chorionic membrane or of the villi, mesenchyme cells with processes in all stages of retraction also were clearly outlined in the homogeneous ground substance. Such evidences naturally remind one of Hofbauer's statement that Marchand called his attention to the fact that these cells were mesenchyme cells, a conclusion which Hofbauer accepted. My implication, however, is not that degeneration of the mesenchyme or of individual mesenchyme cells can proceed only through a Hofbauer stage, but that, especially in the chorionic villi, a form of degeneration of the mesenchyme seems to occur which gives rise to this peculiar cell form, the degenerate character of which rightly impressed Minot. This relationship also attracted the attention of Mall ('15), who represented degenerating villi and stated

The core of the villus gradually breaks down and disintegrates. While this process is taking place we often see scattered through the stroma of the villus large protoplasmic cells. . . . These cells, which I have repeatedly seen in the villi of pathological ova, may be a type of wandering cells; at any rate, when the villus is being invaded by the leucocytes and trophoblast it might be thought that they arise from the latter, but this is improbable.

It is of particular interest in this connection that Virchow ('63) stated that Schroeder van der Kolk ('51) had concluded that large clear cells in the stroma of the villi, later classed among the physaliphores by Virchow, occurred too frequently to be correlated with hydatiform degeneration. This suggests that the so-called Hofbauer cells were known since the early days of cytology, and that some one must have noticed, even at that early date, that they were very common in some hydatiform moles. Whether or not this was van der Kolk I am unable to say, but that Hofbauer cells are especially numerous in some cases of hydatiform degeneration is undoubted. But it does not therefore follow that they constantly are present in this condition. Large numbers of Hofbauer cells occurred in seventeen out of the sixty-one cases of normal and pathologic chorionic vesicles in which they were especially studied. Of these seventeen cases fourteen later were independently identified as showing hydatiform degeneration, and the other three were considered as possibly such. In other words, every case of this series of so-called normal and pathological chorions in which the Hofbauer cells were numerous, was one showing hydatiform degeneration of the villi. It also is true, however, that thirty-four cases containing a few or some Hofbauer cells were not identified as being hydatiform moles, although three cases containing smaller numbers of these cells were so recognized. Moreover, not a single case of this series of sixty-one specimens which contained no Hofbauer cells whatever was later identified as showing hydatiform degeneration.

Somewhat similar evidence was afforded by the study of the twenty-two cases in the protocols of which Mall had noted that Hofbauer cells were present. Of these twenty-two cases, thirteen later were identified as showing this degeneration. However, since a total of 112 cases of hydatiform degeneration were identified among the 313 classed as pathologic among the first thousand accessions in the Mall collection, it is evident that the presence of Hofbauer cells was especially noted in but a relatively small percentage of the series of embryos classed as pathologic. If we include certain other cases in which they came

to attention later, the percentage becomes 26.7; that is, 30 out of 112 cases of hydatiform degeneration. Of these thirty cases containing Hofbauer cells in sufficient numbers to attract attention in the course of a routine examination made for other purposes, seventeen or 56.6 per cent, were later identified as instances of hydatiform degeneration. Since the sixty-one cases in the first series were examined especially for the purpose of study of Hofbauer cells, the higher percentage of correlation observed in this series may be due partly to this fact. At any rate, that such a correlation exists seems to be quite clear, although I do not conclude that the two conditions necessarily or invariably are associated.

It is interesting that Pazzi ('04) considered a dystrophy of the connective tissue with the development of cellular elements "not very well differentiated, but like the plasma cell of Hofbauer," as the initial and pathognomonic change in hydatiform degeneration. Pazzi further stated that the plasma cell of Hofbauer may be in a state of hyperactivity or of degeneration, and questioned the statements that Hofbauer cells appear only at the end of the fourth week and that they have a short life. Pazzi regarded the Hofbauer cell as fundamentally a constituent of the villi, as the decidual cell is of the decidua. He, like Essick, attributed their origin to the endothelium of the vessels, and Pazzi suggested that the Hofbauer cell may have a special internal secretion intended to preserve the stroma of the young villus against degeneration. Pazzi further considered the question whether a Hofbauer cell can transform itself into an epithelial cell and finally into a syncytial cell, adding that the invasion of the stroma of the villus by epithelial growth, is only a special development of Hofbauer cells!

As already stated, Muggia also found these cells very abundant in a case of partial hydatiform degeneration, and held that their appearance and condition was correlated with the proliferation and vacuolation of the syncytium, maintaining that, as the latter becomes vacuolated the lipoid interstitial cells of Acconci appear, the changes in the two being wholly parallel.

Since thirty-two of the fifty-one specimens in this series of sixty-one containing a few, some, or many Hofbauer cells had been classed among the pathologic, it follows that these cells were noticed more frequently in the pathological than in specimens classed as normal. This becomes especially evident if we exclude from this series of fifty-one cases all those containing some or many Hofbauer cells, for of twenty-seven of these, nineteen, or 70.4 per cent, had been classed among the pathologic. Moreover, since the great majority of the conceptuses classed as normal belong among abortuses, one would be entirely justified in questioning the strictly histologically normal nature of the chorionic vesicles which accompany some embryos classed as normal. At any rate, it is evident that the plasma cell of Hofbauer is associated with degenerative changes in the mesenchyme of the villi. Since such changes are more common in pathologic abortuses it is not surprising that Hofbauer cells are more common in the latter than in normal specimens, and, since degenerative changes in the stroma are especially pronounced in advanced cases of hydatiform degeneration, it is still less surprising that Hofbauer cells are particularly common in this condition. But they are not necessarily pathognomonic of hydatiform degeneration, although it is true that when at all numerous they are associated with hydatiform degeneration in about 75 per cent of the cases.

After a careful survey of a considerable number of specimens, both normal and pathologic, ectopic and uterine, of human conceptuses of widely different ages, I am led to concur entirely in the opinion of Minot that the typical vacuolated cell, as described by Hofbauer, is a degeneration product, *though usually not a degenerate erythroblast*, as Minot concluded. Rarely have I seen a chorionic vesicle in which the rather small, clear, isolated Hofbauer cells scattered throughout the stroma of a villus undoubtedly were erythroblastic in origin. In these villi capillaries in various stages of disintegration were present, and the erythroblasts could be traced directly to these degenerate capillaries. In the earlier stages of this degeneration these degenerating erythroblasts are not surrounded by spaces, how-

ever, and this is true also of early stages in the degeneration of the fixed or already detached mesenchyme cell, which later forms the typical, degenerating, wandering cell. However, it represents but one stage in this degeneration.

It is significant that, although Hofbauer suggested that these cells might have a digestive or assimilative function, he, too, frequently found fragmentation of the nuclei and complete disappearance of the cytoplasm and even of the cell itself. All stages of degeneration, as manifested by crenation of both cytoplasm and nucleus, even to complete disappearance of the cell, can easily be found. Signet-ring forms are common, and the nuclei are found in all stages of extrusion and degeneration. The cell boundaries are often ragged, the nuclei crenated and pycnotic, the cytoplasm granular, vacuolated, webbed or fenestrated, until finally nothing but a faint ring or shadow form without a trace of a nucleus remains. However, in these transparent or shadow forms the nuclei, if not previously extruded or dissolved, are frequently represented by a mere outline or by a faint trace of one. Since all stages between the latter and the well-preserved cells, without vacuoles and well-preserved nucleus and cytoplasm, and also with processes, occur in good material, one can scarcely doubt their origin.

Undoubted instances of mitoses were never seen in any Hofbauer cells, no matter how well preserved. This no doubt can be accounted for by the fact that from the time the mesenchyme cells retract their processes and become isolated in the ground substance of the villus, they are in a stage of degeneration. Under such circumstances one would hardly expect to see instances of cell division, although these possibly may be simulated by necrobiotic phenomena.

Hofbauer ('05), and also in his first publication, stated that the cells described by him increase by mitoses which are frequent. He also found examples of what seemed to be instances of pluripolar mitoses, but also noted fragmentation of the nuclei. Acconci ('14) also found mitotic figures in cells designated lipoid interstitial cells by him, but most investigators say nothing about this. On the contrary, a number of them specifically

state that they could not find an actual increase in the number of nuclei present in the stroma of villi containing large numbers of these cells. Furthermore, every one except Muggia (and also he in his description and illustrations, as also Acconci) has noted characteristics, and described the cells in such a way as to suggest the presence of degenerative changes. When at all distinct they are of various shapes and sizes, and are surrounded by a relatively large clear zone. Their occurrence is erratic and they contain lipoid granules or vacuoles, and have nuclei varying considerably in size, position, and staining reaction, as does also the cytoplasm. They are most frequent in degenerate villi and not infrequently lie in detritus. The better preserved the stroma the fewer one finds, and in these observations on this rather large series of chorionic vesicles, some of which were obtained fresh—one living—in hysterectomly specimens, I have only found a few instances of what possibly could be regarded as mitotic figures. Since almost all are agreed that these cells are of mesenchymal or connective-tissue origin, it is easy to see that considerable difficulty must be encountered in deciding just when to regard a mesenchyme cell, which is the precursor, as a Hofbauer cell. However, since I have not made this aspect of the question a particular subject of investigation, I have no other evidence to offer.

Since some of these cells, during the early period of degeneration, after they have become quite circular in outline and the nucleus has taken an eccentric position, have a decidedly granular or even a lumped cytoplasm, the confusion with plasma cells, or their earlier designation as granular wandering cells, need not surprise us. Nevertheless, the term plasma cells is hardly applicable, as many of them are not granular. Moreover, no one has shown that in fixed preparations these cells take the stains specific for plasma cells. Indeed, although he stained material with borax methylene-blue after Jadassohn, Happe ('06) did not find any of the Hofbauer cells impregnated. It must be remembered, however, that failure to stain may be dependent very largely upon the degree of degeneration which the particular cells have undergone, for, as already stated, Hof-

bauer found that in fresh material these cells reacted as plasma cells to vital stains.

The opinion of Minot that Hofbauer cells are degenerating erythroblasts probably can be accounted for by the fact that in the chorionic vesicle from which Minot's series, showing a progressive degeneration of the latter into the former, was obtained, it was impossible to distinguish between the two. This difficulty was due partly to the poor state of preservation of the particular specimen, a larger survey, especially of better material, would have revealed the fact that Hofbauer cells are found in villi, the blood-vessels of which contain no erythroblasts. Moreover, as will appear later, the distribution of these cells in the villi is not such as one rightfully would expect if they have their source in the vessels. However, since the final form of the typical Hofbauer cell is a mere shadow cell, it necessarily may be impossible to determine the kind of cell from which this shadow form arose, for, as is well known, the end forms in process of degeneration of many different types of cells are indistinguishable. Consequently, a group of swollen, highly vacuolated cells also may contain among them degenerated, nucleated red blood cells, as Minot held. Indeed, degenerating erythroblasts which are indistinguishable from some Hofbauer cells, can be seen occasionally not only in the vessels, but in the heart and also within the cavity of the chorionic vesicle; but such occurrences do not prove that the Hofbauer cells of the villi arise from erythroblasts. That this usually is not the case follows also from the fact that well-preserved, non-vacuolated Hofbauer cells occur in villi which have not become vascularized or which, as stated above, no longer contain vessels. It is true that it often is impossible to distinguish between degenerate erythroblasts within the vessels and Hofbauer cells lying outside of, even if near to them, in the stroma of the villus. This difficulty is entirely avoided by examining the older specimens without nucleated reds, for, since Hofbauer cells always are nucleated except in their very last stages, confusion with nucleated cells thus is avoided.

Although the elimination of the erythroblast as the source of the Hofbauer cell was thus very easy, some difficulty was encountered, strangely enough, with regard to polymorphonuclear leucocytes. This largely is due to the fact that the nucleus of the latter often ceases to be polymorphous as the cell degenerates. Instances of this kind are quite common, especially in the membranes of hemorrhagic or infected abortuses. They are, however, also met with in the decidua. Since the polymorphous character of the nuclei of these leucocytes usually can be recognized without difficulty in degenerated accumulations of pus, I was at first predisposed against regarding a circular nucleus as possibly polymorphous in origin, but careful scrutiny of numerous specimens in which these misleading degeneration forms occurred soon left no doubt as to the facts.

As stated above, Hofbauer cells were found in the cavity of the chorionic vesicle in abortuses which contained blood or had become infected. In these specimens the degenerated polymorphonuclear leucocytes usually lie in groups, or more commonly in rows along the inner borders of the chorionic membrane, or in long narrow clefts or folds of the same. Some also were scattered about among the degenerating erythrocytes, but an examination of the contained blood usually surprises one by the entire absence not only of well-preserved polymorphonuclear leucocytes, but of all leucocytes whatsoever. This, to be sure, is in marked contrast to what is found in the case of ordinary hemorrhages and is a fact full of significance for the question under discussion. Most of the degenerated polymorphonuclear leucocytes, many of which contain undoubted evidence of phagocytosis, possess a relatively small, circular, vesicular nucleus which often is eccentric in position. Others are filled with a granular cytoplasm, or even with very discrete golden granules, while still others are filled with dark, black pigment granules corresponding in size to the golden ones. Here and there the field of degenerating erythrocytes may also be studded with masses of pigment which clearly declare their origin by the presence of all manner of transition forms, between the well-preserved, easily recognizable polymorphonuclear leu-

cocytes and the disintegrated pigmented detritus. The phagocytic nature of these cells is especially noticeable in the specimens of young chorionic vesicles with nucleated reds, stained with iron hematoxylin, for in these the leucocytes are often seen filled with a mass of nuclei only.

Similar appearances can also be seen occasionally in the decidua from cases of endometritis, as well as in portions of the decidua in which the glands have undergone considerable maceration and degeneration. In the former the polymorphonuclear leucocyte is the misleading form, while in the latter the degenerating, cast-off glandular epithelial cells simulate Hofbauer cells in almost every morphological detail. I have also seen similar specimens of degenerated polymorphonuclear leucocytes in ill-preserved hemorrhagic lymph nodes, especially from cases of septicemia, and, until the true nature of such degenerate leucocytes became evident, it was very puzzling to see why the Hofbauer cell, which never was found to contain evidences of phagocytosis when lying in the stroma of the villus, should become phagocytic when contained in a degenerated amniotic or chorionic membrane or when lying in a hemorrhagic area. Undoubted instances of phagocytic Hofbauer cells were never seen, although certain misleading forms other than those already mentioned were encountered also in pregnant tubes and in an ovarian pregnancy. Among these misleading forms were specimens of binucleate cells in which one nucleus had undergone almost complete chromatolysis, leaving only a nuclear membrane. These nuclear remnants or so-called nuclear shadows, can easily simulate a phagocytosed erythrocyte. The same is true of small areas of cytoplasm which stain but faintly, and hence look more translucent, and particularly of vacuoles themselves.

Essick ('15) found what he regarded as morphologically similar cells in transitory cavities in the corpus striatum, and believed them be macrophages. Consequently, he concluded that Hofbauer cells also are phagocytic and regarded them as having an endothelial origin. I have not been able to find any evidence for the latter origin, however, for in specimens in which the capillaries are plugged with degenerate endothelial cells

or in which they are composed of a layer of greatly enlarged oedematous endothelial cells, so as to make the cross-section of the vessels look not unlike that of a duct, Hofbauer cells never were found in close proximity to capillaries or other vessels or in unusual numbers elsewhere in the stroma of such villi. Nor did I see any evidence for such an origin in villi from hemorrhagic or inflammatory cases, and although Hofbauer cells often lay near to, or even in extravasations in the villi, they never were found engorged with erythrocytes or pigmented. Nevertheless, if Hofbauer cells arise from mesenchyme cells, it stands to reason that they at least may be potentially phagocytic, and failure to find them so may be accounted for by the fact that they possess a lowered vitality in consequence of degenerative changes.

I am prompted to suggest, in connection with the question of phagocytosis, that, unless we regard the process as other than an actively vital movement on the part of the cell for the purpose of engulfing things, we have undoubtedly misused the term. That the mere possession of parts of cells, or even of whole cells within the cytoplasm, is not sufficient evidence for the possession of phagocytic activity on the part of a particular cell, seems to me to be beyond question. In some instances, for example, degenerating phagocytic leucocytes fuse with each other in groups of twos, threes or even in greater number, thus forming multinucleated and not infrequently vacuolated complexes. Similar phenomena can be seen also among degenerated erythroblasts and trophoblast cells. Although it would be incorrect to regard these degenerate fusion products as true, living giant-cells, they nevertheless simulate such very closely indeed. Moreover, when these larger fusion products fuse with an individual cell of the kind that gave rise to them, it would be quite natural to regard them as being phagocytic, while, as a matter of fact, the process is merely one of degeneration. Another example of what we may call pseudophagocytosis is that represented by the isolated erythroblasts rarely seen in the stroma of a villus. In some instances two or three cells, whose boundaries for the most part still are clearly outlined, can be seen to

have partly fused, forming a so-called giant-cell. All transition forms and stages can be found, and were it not for this fact, the resultant large multinucleated fusion product, if seen to join with an isolated trophoblast cell, might be regarded as being phagocytic. Other instances of a similar nature were discussed briefly elsewhere (Meyer, '18), and I am inclined to believe that the non-vital character of this kind of cell formation, which occurs under conditions of cell degeneration, needs further emphasis. It certainly would seem to be a non-vital, rather than a vital phenomenon. It is indicative of degeneration and death, rather than of regeneration and life.

Cells which are morphologically identical with certain stages in the degeneration of the Hofbauer cell can also be found in entirely different locations than those mentioned. Such instances occur in the Graafian follicle. In some of these, germinal epithelial cells which have become detached and displaced in the liquor folliculi become swollen and transparent and the nucleus takes an eccentric position. In all details of structure and ordinary staining reaction, as shown by hematoxylin and eosin, by iron hematoxylin, by van Gieson, and by Mallory, these cells are identical with phases in the typical Hofbauer cells. This, however, does not justify us in designating them as such, unless we wish to extend the use of this name to degenerating and disintegrating forms of cells of very many different types and origins.

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Resumido por el autor, A. R. Ringoen.

El desarrollo de las glándulas gástricas de *Squalus acanthias*.

Los primeros vestigios de las glándulas gástricas pueden discernirse en el epitelio estomacal de los embriones de *Acanthias* de 133 mm. de longitud. Dicho epitelio se caracteriza en este estado por una gran actividad que se traduce por cambios locales. Grupos de células epiteliales presentan cambios en su reacción hacia el colorante empleado (hematoxilina férrica-naranja G). Tan pronto como una célula epitelial entra a formar parte de uno de estos grupos, tanto el citoplasma como el núcleo disminuyen su afinidad con el colorante citado. En los embriones de 137 mm. de longitud el epitelio gástrico está sembrado de grupos celulares bien definidos, separados por intervalos regulares. Tales grupos no están ya formados por células epiteliales sino que representan los rudimentos de glándulas. Su diferenciación ulterior comprime los extremos libres proximales de las células del epitelio. A consecuencia de esta compresión, las células situadas entre dos rudimentos glandulares adquieren una disposición en abanico cuando se observan en corte transversal. Los rudimentos glandulares crecen y penetran en el tejido mesodérmico subyacente produciendo las glándulas gástricas. A medida que estas invaden el tejido mesodérmico las células que las constituyen sufren una rotación, al final de la cual, las del fondo de la glándula se colocan con sus ejes mayores formando un ángulo recto con la luz glandular. Una glándula gástrica de *Acanthias* completamente desarrollada es una estructura no ramificada. La diferenciación de dos tipos celulares no se lleva a cabo nunca.

THE DEVELOPMENT OF THE GASTRIC GLANDS IN *SQUALUS ACANTHIAS*

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SEVEN FIGURES (THREE PLATES)

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INTRODUCTION

The digestive tract of fishes, although the subject of a considerable amount of research, still affords opportunity for further study. The very early investigators of the subject were interested in its glands, but no serious attempts were made to determine their origin and further differentiation. A study of the literature shows that there is still comparatively little written on the histogenesis of these structures. In view of these facts, the present paper attempts to elucidate the sequence of events that takes place in the development of the gastric glands as found in *Squalus acanthias*.

The material used in this study was placed at my disposal by the Department of Anatomy, University of Minnesota. For the study of the glands, the stomach was removed from the

specimen, embedded in paraffin, and cut in serial sections 5 μ in thickness. The sections were stained with iron-haematoxylin. Both erythrosin and orange G were employed as counterstains. For a large part of the prepared *Acanthias* material I am personally indebted to Dr. Richard E. Scammon, who has so kindly permitted me to use numerous series from his private collection (S. C.).

It is with great pleasure that I express my appreciation to Dr. Scammon for suggesting this investigation, for the loan of material, and for the interest shown during the progress of this study. I also wish to express my indebtedness to Mrs. Helen Sanborn Chapman for the accurate drawings.

LITERATURE

Our conceptions of the histogenesis of the glandular elements of the digestive tract are based largely on observations made on the study of mammalian material. A comprehensive review of the literature bearing on the subject would be foreign to the purpose of this study. Later an attempt will be made to indicate the present status of our knowledge in this field, in so far only as it may be essential to a clearer understanding of gland formation in *Squalus acanthias*, by brief reference to a few papers.

Sprott Boyd ('36) made the first observations on the presence of gastric glands in mammals and fishes. Following Boyd's observation, Bischoff ('38) studied the mucous lining of a great many species of fishes. In some species he was unable to find glands, while in others they were abundant.

In 1852 Leydig discovered gastric glands in *Squatina angelus* and *Torpedo galvani*. Later he referred to these glands as 'Labdrüsen,' thinking that they were comparable to the gastric glands of mammals.

Edinger ('77), in studying the mucous membrane of the stomach of fishes, was unable to distinguish the chief and central cells as discovered by Heidenhain and Rollet in the mammalian stomach. According to Edinger, among the Teleosts there are a number of forms that possess no glands in the stomach.

He believes that the gastric glands appear phylogenetically first in the selachians.

The literature on the glands of the Selachii has been to a large extent reviewed by Oppel ('96), but one finds very little information relative to the origin of the gastric glands themselves.

Sullivan ('07), in the course of a study devoted to the digestive tract of Elasmobranchs, considers primarily the physiological features of the glands. He makes no comment upon the development of the glands.

Peterson ('09) is, as far as I am able to ascertain, one of the few investigators to consider in some detail the development of the gastric glands in selachians. In his studies on the histogenesis of the glands in a number of these forms he describes and figures epithelial outgrowths as the rudiments of glands.

OBSERVATIONS

In *Squalus acanthias*, gland development proceeds in a very different manner from that commonly described for a number of mammals, including man. The conditions in the gastric epithelium of embryos 133 mm. in length indicate that in the selachians gland formation is not associated with the formation of gastric pits, as has been claimed for man. Since *Acanthias* specimens of this length show the first traces of gland differentiation, I begin with this stage and follow out in later stages the complete evolution of a gland.

A. Early changes in the gastric epithelium

The early stages in the development of gastric glands are clearly followed in *Squalus acanthias* embryos 133 mm. long. In fact, the very beginnings of the glands are discernible here as differentiating in the gastric epithelium itself. At this particular stage the epithelium of the stomach is characterized by its great activity in the way of undergoing definite local changes throughout its entire extent. Prior to the 133-mm. stage there were no apparent variations or irregularities in it;

all of its constituent cells presented similar morphological features and identical staining reactions. The nuclei also presented their own characteristic configuration and staining reactions. With the establishment, however, of local changes in the gastric epithelium (133-mm. embryos) there appear certain definite modifications in its cellular make up. Figure 2, from a 133-mm. embryo, shows a number of these modifications. The two cells on the extreme right and left, respectively, of this figure represent the typical columnar epithelial cells so characteristic of the selachian stomach. They are long and narrow, and are without a basement membrane. The cytoplasm stains a faint grayish tint with iron haematoxylin-orange G. The nuclei are long and slender; they present a deeper gray tint than does the cellular cytoplasm. The four cells just described are destined to remain as epithelial cells. Interpolated between, however, are a number of other cells which are characteristically different from the former in both their cytoplasmic and nuclear staining reactions. A number of these cells may still show a close relationship to the neighboring epithelial cells as exemplified by their behavior toward the iron haematoxylin-orange G stain. In most cases, however, the cytoplasm and the nucleus do not stain in the same manner as in the adjacent epithelial cells. The whole tendency of staining variations in this direction is for an epithelial cell to decrease in its avidity for the stain as soon as it is called upon to assume a different rôle from that of an ordinary epithelial cell. At times such a change in the staining reactions of the cell body may precede somewhat that of corresponding changes in the nucleus or vice versa, and again the changes may proceed rather synchronously in both the cytoplasm and the nucleus.

In addition to the above-described staining reactions, there is also a further change in the form of the nucleus, as shown in figure 2. On the right-hand side of the figure, in the third cell from the margin, is a nucleus which has not changed its staining reactions, although the cell body has progressed to some extent in that direction. It is apparent from the figure that the nucleus is undergoing a change in its shape. No longer

does it correspond in its configuration to the long narrow epithelial nucleus contained in the cells at the extreme right and left of the figure. A decided change in shape is clearly seen in those cells which have changed both their cytoplasmic and nuclear staining reactions.

If the conditions in the gastric epithelium of 133-mm. *Acanthias* embryos, as I have described them in figure 2, are interpreted properly, they would indicate that in this group gland development is of a rather primitive nature. No specialized cells are set apart in early embryonic life for the origin of glands, but the general epithelium is endowed with the capacity of transforming certain patches of its constituent cells into definite gland rudiments at the proper time. When the proper time is at hand, small groups of these apparently similar epithelial cells change their staining reactions and the shape of their nuclei, and differentiate in another direction. They continue to evolve in a very definite direction, because they are incapable of giving rise to any other structure than the particular gland rudiment towards which their potentialities are directed.

B. Formation of definite gland rudiments

The characteristic changes which appear in the gastric epithelium at the 133-mm. stage are even more pronounced in slightly longer embryos. In 137-mm. specimens the epithelium has been modified only at those points where glands are to be formed. It is surprising with what regularity the apportioning of the general epithelium into glandular and non-glandular areas has taken place at this stage. The number of epithelial cells which intervene between two potential gland areas is practically the same in all cases. Just what factors determine the selection of certain groups of epithelial cells as the precursors of glands to the absolute exclusion of others obviously can no more be answered than why certain entodermal cells will differentiate into liver cells, while other similar cells, at least so in their early stages of differentiation, will give origin to pancreatic tissue.

On comparing figures 2 and 3 (the latter an embryo of 137 mm.), it is apparent that gland rudiments are now sharply marked off from the neighboring epithelial cells. In embryos of 137 mm. in length, such areas are very numerous. The entire epithelium of the stomach is literally studded at regular intervals with them. At this time none of the cells making up a gland rudiment present staining characters bordering on those of the epithelial cells. The nuclei present about the same staining reactions as portrayed in figure 2. There has been, however, a considerable progressive change in their shape.

Figure 3 shows two gland rudiments embedded in the epithelium. They present such a striking appearance in sections of the stomach (137-mm. specimens) that one cannot fail to notice them. The regularity in their distribution is indeed striking. Never have I seen similar rudiments lying at the base of the epithelium. Obviously, there would be no reason for such a location, since the early gland rudiments are simply transformed epithelial cells. In the same figure the two gland cells at the extreme right represent only a portion of a gland rudiment, due to the plane of sectioning.

That the potentialities of the epithelial cells are by no means the same is particularly evident in the gastric epithelium of *Acanthias* specimens 137 mm. in length. Every gland of the adult specimen is represented at this stage by its own epithelial modification or gland rudiment.

C. Influence of the gland rudiments on the epithelium

The differentiation and presence of the gland rudiments in the epithelium has had a profound influence on the final configuration of the epithelium itself. During the early stages of differentiation it increases in thickness; at no time is it stratified, although in the early stages of embryonic development the disposition of the nuclei in several planes simulates stratification. In *Acanthias* embryos 133 mm. in length, the epithelium consists of columnar cells with their lateral surfaces closely approximated (fig. 2). The differentiation and pres-

ence of gland rudiments have changed this simple uniform condition. No longer are the epithelial cells arranged in the form of a single continuous row in embryos 137 mm. long. On comparing figures 2 and 3, it is evident that as soon as a gland rudiment is well marked out in the epithelium (fig. 3) it forms a bottle-like plug. The lower expanded portion of the plug tends to press upon the neighboring epithelial cells, thus crowding them closer and closer together. This lateral displacement of the epithelial cells proceeds unhampered, as they are not anchored fast by a basement membrane, but may extend freely down into the underlying mesodermic tissue.¹ As a result of compression exerted by the expanded part of the bottle-like plug of the gland rudiment, or for a lack of space, the epithelial cells are closely approximated at their bases. Since the distal parts of the epithelial cells have not been affected by the mechanical forces involved in the compression phenomena, it naturally follows that the portion of epithelium intervening between two gland rudiments takes on a fan-shaped form in cross-section (fig. 3). This peculiar arrangement of the epithelial cells, as compared with the simple uniform condition in the earlier stages, is maintained in the later stages.

D. Subsequent history of the gland rudiments

The subsequent history of the gland rudiments will now be considered in detail. *Acanthias* embryos 137 mm. long furnish very favorable material for such considerations. At this stage many of the gland rudiments are undergoing a great change in their length; their constituent cells are growing out into the mesodermic tissue. During the course of a very short period of time every gland rudiment will have elongated and burrowed its way into the underlying tissue.

Figure 4 represents one of the so-called epithelial outgrowths in the gastric epithelium of an *Acanthias* embryo 137 mm.

¹ Hopkins ('95) believes that a basement membrane does not exist in the ganoids. According to Edinger ('77), fishes possess no basement membrane, but the epithelium borders directly upon the underlying tissue.

long. At this stage the constituent cells of a gland rudiment, and as illustrated in figure 3, have simply elongated and are pushing their way out into the underlying mesodermic tissue. During the pushing and burrowing of an outgrowth down into the mesodermic tissue there is an actual migration of its cells. Now the nuclei have increased greatly in size, as compared with those shown in the gland rudiment of figure 3, and at this stage they tend to occupy the most distal parts of the outgrowth. No doubt the increase in the size of the nuclei at this time—preparatory to division—is associated with the fact that the cells which contain them are to grow out still further at a subsequent time, and obviously this requires additional cells (see fig. 5, also from a specimen 137 mm. long, for documentary evidence on the further growth of a gland rudiment; increase in the number of nuclei with a distinct change in their size and shape, as compared with fig. 4). Cell outlines are just as distinct here as they were before the gland rudiment cells began to grow out into the mesodermic tissue (compare fig. 2 with fig. 3).

In slightly more advanced stages of differentiation than is represented in figure 4, an outgrowth becomes more or less tubular in form. This condition is represented in figure 5, also from a 137-mm. specimen. With still further differentiation, as I shall attempt to show at a subsequent time, this simple condition is radically changed.

Another point of considerable interest in connection with figure 5 is the position of the nuclei in the tubular outgrowth. Those located in the region where the gland rudiment first burrowed do not seem to present any definite arrangement in their distribution within the outgrowth. On the other hand, most of the nuclei which are found in the lower half of the outgrowth show that their long axes are placed parallel to the long axes of the tube. In slightly more differentiated outgrowths this parallel arrangement is decidedly modified. Even as shown in figure 5, there is a slight tendency in this direction, for several of the nuclei are on the verge of shifting their axes.²

² The nuclei, as will be pointed out later, are not actually changing their long axes, but the cells containing them are shifting.

During the extension of a glandular outgrowth down into the mesodermic tissue it meets with a small amount of resistance for two reasons. In the first place, the constituent cells of an outgrowth are not anchored fast by any structure comparable to a basement membrane, and in the second place, the mesodermic tissue is of such a loose character that it is simply pushed back and more or less condensed by the burrowing cells (figs. 4, 5, 6, and 7).

E. Rotation of cells

Figure 6, from an embryo 137 mm. long, shows a glandular outgrowth at its maximum length. On comparison with figure 5, it is apparent that the staining reactions of both the nuclei and cytoplasm are identical. The morphological features of the nuclei are also the same. The glandular outgrowth is no longer a tubular structure; the shape of its proximal end has been characteristically modified. This change of form may be ascribed to a rotation of cells in the lower two-thirds of the outgrowth. Although cell boundaries are not distinguishable in this figure, it is apparent on comparison with figure 5, in which instance cell boundaries are also absent, that in following the course of the nuclei in figure 6, as compared with those shown in figure 5, one is simply tracing out the movements of the cells that contain them. A number of cells, as represented in figure 6, have rotated through an angle of about 90° . The completion of the rotation process is seen in figure 7 (from a specimen 146 mm. long). In this stage cell boundaries are evident; the long axes of the nuclei, instead of being placed parallel to the long axes of the future gland, as they were in the tubular outgrowth represented in figure 5, are now placed at right angles to the lumen of the gland. Scammon ('15), in the course of a study devoted particularly to the histogenesis of the selachian liver, describes a similar rotation of cells in tubule anastomosis. He does not think that the nuclei shift their axes within the cells, for he frequently finds, in cases where faint cell boundaries can be made out, that the cells show the same changes in position as do the nuclei.

In order to elucidate more clearly the sequence of events that takes place in the evolution of a selachian gland from the simple tubular condition on through to the typical flask-shaped form, it has seemed feasible to submit a number of diagrams illustrative of the rotation processes. A casual reference to these diagrams will enable one to comprehend at a glance, without the aid of detailed figures and descriptions, that an actual rotation of cells play a fundamental rôle in molding the fundic portion of the Selachian gastric gland.

In figure 1, diagram A, the glandular outgrowth has extended only a short distance, and in most instances the long axes of the nuclei are parallel to the long axes of the outgrowth. This is the condition one would expect to find, since in a slightly younger stage (fig. 3) the nuclei are also parallel to the long axes of the gland rudiment. Diagram 1B shows that the glandular outgrowth has not only increased in length, but that a number of the nuclei are beginning to change their long axes with reference to the long axes of the outgrowth. With reference to these shifting movements Scammon ('15) states "that it is hardly to be considered that the nuclei shift their axes within the cells." Since cell boundaries were not seen in the specimen, illustrated in figure 1B, it may be reasonably assumed that the shifting movements of the nuclei are but the shifting movements of the cells which contain them. The next diagram (fig. 1C) distinctly shows that the cells in the region of what is to form the future fundic portion of the gland have rotated through an angle of about 90° . It is very probable that the cells located at what may be called the center of the base of the fundus do not change their positions, since their nuclei show no shifting movements at any time. Diagram 1D shows that all of the nuclei have passed through a change in their original axes of about 90° . The long axes of the nuclei now lie at right angles to the lumen of the gland. In figure 7 the outlines of the cells were drawn just as they appeared in the actual specimen. This shows that the rotation of the nuclei is to be interpreted only as the shifting movements of the cells that contain them.

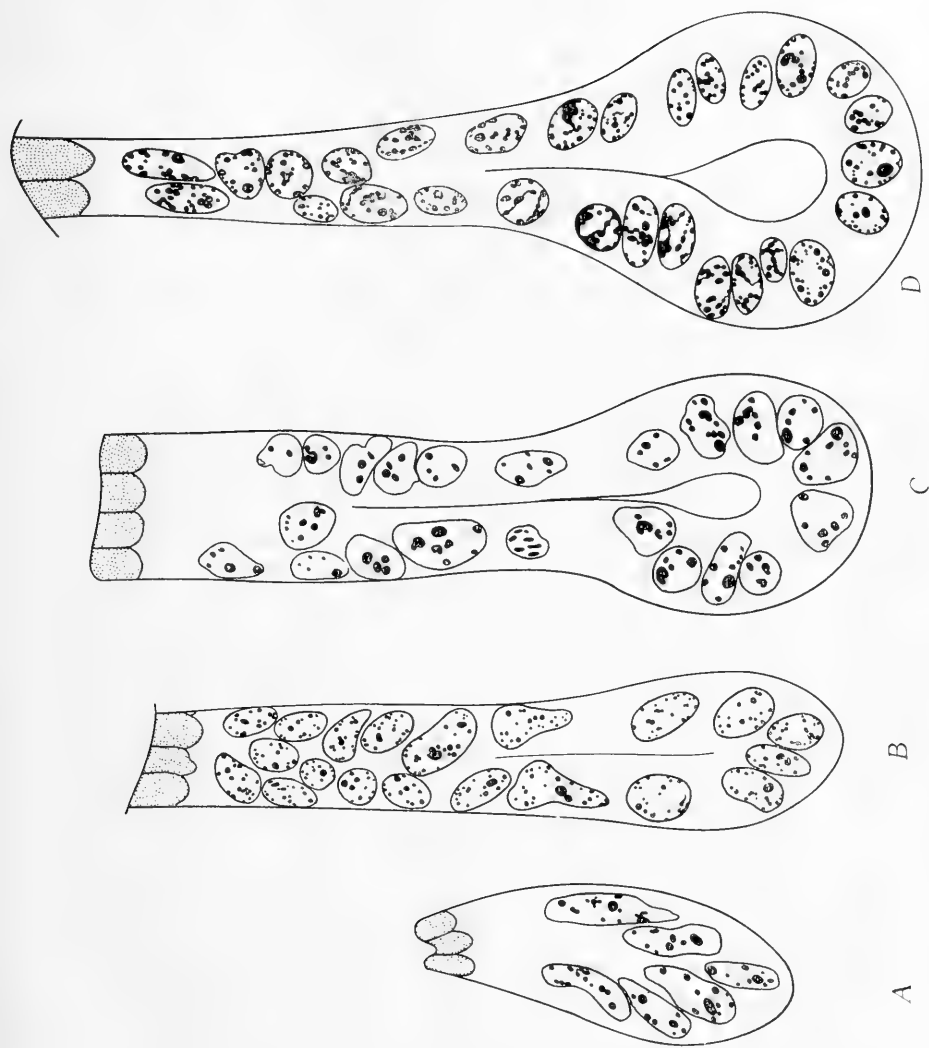


Fig. 1 Diagrams showing four stages in the evolution of a selachian gastric gland. All of these diagrams are drawn from actual specimens. All of the nuclei were outlined with the camera lucida and drawn with the same magnification, $\times 800$. 1A is drawn from an embryo 133 mm. in length; 1B and 1C from embryos 137 mm. in length, and 1D from a 146-mm. specimen.

F. Histology of the fully differentiated gastric gland

Figure 7 (embryo 146 mm. long) represents the fully differentiated gastric gland. In specimens of this length the vast majority of glands present such an appearance, although there may be slight variations in the length of the neck and in the size of the fundic portion. These slight variations are, however, of no fundamental importance, for they do not signify that the glands differ in character.

All of the gastric glands are unbranched, flask-shape structures. Never have I been able to find the bifurcated forms described by Peterson('09). He believes that the neck cells of the gland are of a rather primitive character during their early history, and that they may give origin to a side bud which with further differentiation, gives to the gland a forked appearance.

At no time have I been able to distinguish more than one type of cell in a gland (fig. 7). All authors who have investigated the gland cells of lower vertebrates are quite agreed in the occurrence of a single cell-type.³ According to Edinger ('77), in the fishes, this single type is homologous neither to the chief nor to the parietal cell of the mammalian stomach. His conclusion has been generally accepted. It appears from the investigations of numerous other observers that the differentiation of two the cell-types in the gastric glands of fishes does not obtain. This specialization probably appears phylogenetically much later.

As far as *Squalus acanthias* is concerned, and as Peterson ('09) maintains for a number of selachians, the neck cells of a gastric gland are not concerned in the formation of any specific secretion. This activity appears to be confined to the cells making up the fundic portion of the gland. In all the cases that have come under my observation, I have found that the secretion granules are elaborated exclusively in these cells. As to the precise character of these granules, I have no definite knowledge.

³ Oppel ('96) agrees with Edinger in the finding of only one type of cell in the glands of fishes, but states that this single type appears to possess relationships with the parietal cell of mammals.

DISCUSSION OF RESULTS AND LITERATURE

Miss Ross ('02), in her investigations on the development of the gastric glands in *Desmognathus*, *Amblystoma*, and pig, finds that the round granular cells which give origin to the glands appear as distinct cells with the differentiation of the entoderm. These gland cells occur at the base of the epithelium in close contact with the basement membrane.

In addition to Miss Ross ('02), Toldt ('81) also maintains that the beginnings of the gastric glands in the cat are represented by granular cells, which are interpolated at the basal parts of the epithelial cells. There are, however, numerous other investigators who ascribe the origin of glands to epithelial projections, or outgrowths,⁴ among whom may be mentioned Kölliker ('52), Brand ('78), Griffini and Vassale ('88), Oppel ('96), Minot ('02), and Peterson ('09). Johnson ('10) finds in a human embryo of 120 mm. that the beginnings of the gastric glands appear as knob-like outgrowths at the bottoms of the gastric pits. It is generally admitted by those who believe that the glands arise from downgrowths of the surface epithelium, that the gastric pits—later elongated to form grooves—give origin to the glands in this manner.

In specimens of *Squalus acanthias* there are no gastric pits; the longitudinal folds, which are presumably the forerunners of the villi in higher forms, are variable in their number, location, and size. In all probability, their presence is due to the contraction of the muscular coats. Consequently, gland development must go on in a very different manner from those forms where all these features can be readily identified. Peterson ('09) states that in a number of selachians he has found that they arise from epithelial outgrowths directly. With regard to the origin of the outgrowths themselves, and their further history as based more particularly on a study of *Acanthias vulgaris*, he states:

⁴ Bensley ('00) finds in a *Urodele* larva 11 mm. in length that the oesophageal glands also appear as tubular downgrowths of the foregut entoderm. He believes that these glands represent gastric glands whose development has been arrested.

. . . . es erreichen nicht alle Zellen die freie Oberfläche, sondern viele bilden eine untere Schicht und entbehren des Pfropfes. Diese lassen die Magendrüsenzellen aus sich hervorgehen. Sie wandern aus, ein kurzer, halbkugeligter Fortsatz ragt aus dem Epithel heraus, vergrößert sich, der Kern rückt nach. Andere dicht daneben liegende tun dasselbe, so dass eine Knospe an der Epithelbasis zum Vorschein kommt. Die Zellen bleiben mit einem lang ausgezogenen Ende zunächst noch mit dem übrigen Epithel in Verbindung. Immer mehr Zellen rücken nach und drängen die erst ausgewanderten weiter

Since I have already discussed the origin of gland rudiments in *Squalus acanthias* specimens, no further attempt will be made here to consider them in detail. Suffice it to say that in specimens 133 mm. long, gland rudiments differentiate in the gastric epithelium itself—not from special embryonic cells set apart with the early differentiation of the entoderm (as Miss Ross ('02) maintains for *Desmognathus*, *Amblystoma*, and pig),—but from typical epithelial cells (fig. 2).

It may be remarked that Peterson's observation that in *Acanthias vulgaris* embryos of 55 to 70 mm. in length not all of the epithelial cells reach to the free surface—lumen side of the stomach—"sondern viele bilden eine untere Schicht und entbehren des Pfropfes. Diese lassen die Magendrüsenzellen aus sich hervorgehen . . . ,", is not in accordance with what I find in the epithelium of *Squalus acanthias*. For it is clearly seen in embryos of less than 133 mm. in length that all of the epithelial cells at this stage present the closest morphological features and staining reactions. They are all of equal length, and, therefore, they all reach to the surface.⁵ Even

⁵ In support of my results I wish to quote from Kirk's ('10) paper with particular reference to Toldt's ('81) work on the rudiments of glands in the fundic portion of the cat's stomach as large, eosinophilic cells interpolated at the base of the surface epithelial cells. "Toldt is sure these cells are of epithelial origin, but believes they at no time reach the surface, being always shut off from the latter by the overhanging distal ends of the tall pyramidal surface epithelium; he suspects that they arise from young Ersatzzellen. His Ersatzzellen have almost certainly been shown by the work of Stöhr (1882) and Bizzozzero (1888) to be 'Wanderzellen.' Griffini and Vassale maintain that Toldt's figures and text harmonize remarkably with their findings, except that Toldt, through use of oblique sections, erroneously concluded that these primary gland cells do not reach the surface, and that their lumen is thus not at first continuous with the

at the 133-mm. stage, and as shown in figure 2, all of the epithelial cells still reach to the surface. There are, however, considerable changes in the staining reactions of small groups of them. These changes are very significant, for they show that, in spite of their morphological similarities, not all of the epithelial cells are endowed with the same specificities for further differentiation. Small groups of them will remain as epithelial cells, while other groups change their staining reactions, and the shape of their nuclei, and finally form a bottle-like plug which is embedded in the epithelium (fig. 3). All of these changes were considered in detail in connection with figures 2 and 3.

Since Peterson maintains that the gastric glands grow out from portions of the epithelium whose cells do not reach the surface, it would be interesting to know how he would account for certain nuclear variations. In figure 5 the nuclei are numerous in the glandular outgrowth. On comparing one of them with the epithelial nucleus on the left-hand side of the same figure it is apparent that there are fundamental differences with reference to their form, and also in the amount and distribution of their chromatin material. These differences were also mentioned in connection with figures 2 and 3. How would Peterson explain such differences? As far as the nuclear differences in figures 2 and 3 are concerned, he would be unable to give any logical explanation, because he failed to find similar stages in his material. Now, if the gastric glands in Selachians really arise by means of epithelial outgrowths, as Peterson holds, one would naturally expect to find similar nuclei in both the outgrowths and the general epithelium. Figures 4 and 5, however, show that this is not the case. They are very different in their form, size, and in their arrangement of chromatin material. This is precisely what one would anticipate after studying stages similar to the ones depicted in figures 2 and 3 of this paper.

stomach lumen. Griffini and Vassale found many such groups with lumina apparently shut in on all sides, but reconstructions always demonstrated continuity with the stomach lumen from the first."

With reference to the formation of the gland lumen, I find that it arises in the manner described by Peterson. The early formation of it is frequently seen as a slight indentation on the free surface of a well-defined gland rudiment, shortly after it has pushed out into the underlying mesodermic tissue. The further extension of the gland rudiment is followed by a similar extension of the gland lumen. As to whether the extension of the lumen into the fundic portion is responsible for the shifting movements of the cells, I am unable to say. At all events the extension of the lumen into the fundic portion of the glands does not cause the cells there to take on a 'schräge' position with reference to the lumen, as Peterson maintains. I have already pointed out that in the fully differentiated gastric gland the cells at the fundic portion are placed at right angles to the lumen of the gland with reference to their long axes.

In spite of his observations relative to the origin of gastric glands in selachians, Peterson is at a loss to account for the great numbers of these structures that he finds in old specimens. To quote from his paper:

Wie erfolgt nun die weitere Vermehrung der Drüsen? Bei einem jungen Tiere hat die Schleimhaut dasselbe Aussehen wie bei einem alten. Es kommen genau so viel Drüsen auf den Quadratmillimeter; auf einer Schnittstrecke von 1 mm. (Schnittdicke $10\ \mu$) liegen quer- wie längsgeschnitten 20 Schnitte im Durchschnitt, also 400 auf den Quadratmillimeter. Ein Magen eines jungen Tieres (Magenlänge 5 cm.) habe n qmm Fläche, der eines alten (Magenlänge 10 cm.) sagen wir dann $4\ n$ qmm Fläche, so kommen auf den einen Magen 400 n , auf den anderen 1600 n Drüsen, wo kommen diese 1200 n weiteren Drüsen her?

In order to account for these 1200 new glands Peterson thinks that there may be a longitudinal splitting of the young glands. As a result of this cleavage process there are now two glands where before there was only one. He also thinks that "Verzweigungen der Drüse in den mittleren Partien könnten als Anhaltspunkt dienen" for increasing the numbers of the glands. Furthermore, Peterson thinks that the neck cells of a young gland may contribute to the formation of additional glands: "Sie haben wir oben als indifferente Reste der alten Anlage

kennen gelernt, und sie könnten also auch späterhin Drüsenknospen entwickeln. Die anderen Zellen der basalen Reihe sind vollkommen verschwunden—aufgebraucht." He states, however, that he has no proof for such activity on the part of the neck cells.

Peterson's statement, "bei einem jungen Tiere hat die Schleimhaut dasselbe Aussehen wie bei einem alten," is absolutely not in accordance with my observations. Figures 2 and 3 show that there are decided morphological and staining differences, and in these specimens, from which the drawings were made, there is only a difference of 4 mm. in length. In figure 2 (embryo 133-mm. long) the epithelium is undergoing definite changes, while in figure 3 (137-mm. long) it shows more decided changes—the rudiments of glands.

It is unnecessary to build any elaborate theories with reference to the manner in which the number of glands is increased, as Peterson has attempted to do. Any attempt with such an aim in view is diametrically opposed to what one finds in the gastric epithelium of *Acanthias* specimens 137 mm. in length. At this stage the epithelium has been modified at only those points where glands are to be established, and, indeed, it is surprising with what regularity and precision these changes occur. The entire epithelium is literally studded at regular intervals with them. Every gland of the adult animal is represented at this stage by its own epithelial modification, or gland rudiment. In specimens 146 mm. long and above this length, all the rudiments have given origin to glands, and the neck portion of every gland occupies the same relative position in the epithelium as did the early gland rudiment. The entire process of glandular development takes place in just as orderly a manner as does the differentiation and development of any other vertebrate organ or structure.

Peterson's entire difficulty in failing to be able to account for the vast numbers of glands in old specimens is easily explained. Although he saw numerous epithelial buds ('Knospen') in young specimens, they were by no means sufficiently numerous to account for all the glands in the adult specimen.

Undoubtedly the specimens that he examined showed comparatively few buds. I have also frequently found that in embryos 137 mm. in length they are not especially numerous. Therefore, since Peterson did not discover the precursors of the buds themselves, or the gland rudiments as I have called them throughout this paper, he is tempted to theorize with reference to the manner in which the number of glands is increased.

SUMMARY

1. In the selachians, as represented by *Squalus acanthias*, no specialized cells are set apart in early embryonic life for the formation of glands (as described by Miss Ross for *Desmognathus*, *Amblystoma*, and pig), but the general epithelium is endowed with the capacity of transforming certain groups of cells into definite gland rudiments.

2. The apportioning of the gastric epithelium into glandular and non-glandular areas is evident in *Acanthias* embryos 133 mm. in length. At this stage the epithelium is undergoing local modification in that small groups of its constituent cells change their staining reactions (iron haematoxylin-orange G) as compared with the adjacent epithelial cells (fig. 2). As soon as an epithelial cell is called upon to become a contributory member toward the formation of such a group of cells, both its cytoplasm and nucleus decrease in their avidity for the above-mentioned stain.

3. In addition to this change of staining reactions, there is a further change in the morphology of an epithelial nucleus. Many a nucleus is seen in the process of changing its shape from the typical narrow, elongated type, so characteristic of the young epithelial cell, to the plump nucleus of a gland rudiment cell.

4. That the potentialities of the epithelial cells are by no means the same is particularly evident in the gastric epithelium of *Acanthias* specimens 137 mm. in length. At this stage the epithelium is studded at regular intervals with well-defined groups of cells. These are no longer to be considered as epithelial cells, but the rudiments of glands (fig. 3).

5. The presence and further differentiation of the gland rudiments in the epithelium play an important part in causing the proximal free ends of the epithelial cells to be laterally compressed. As a result of compression, the epithelial cells intervening between two gland rudiments are forced to take on a fan-shaped arrangement in cross-section (fig. 3).

6. *Acanthias* embryos 137 mm. in length show that many of the gland rudiments are growing out into the underlying mesodermic tissue to form the future gastric glands (figs. 4, 5, and 6). In the course of a very short time all the gland rudiments have given origin to glandular outgrowths. Peterson recognized similar outgrowths, but he thinks that they arise from certain epithelial cells which do not reach to the free surface—lumen side of the stomach. His interpretation is obviously incorrect because, on the basis of it, he is unable to account for all the glands in older specimens. Peterson failed to discover stages similar to those shown in figures 2 and 3, and these are the critical stages in establishing the number of glands for any given specimen.

7. As the glandular outgrowths invade the mesodermic tissue there is an actual rotation of their cells (fig. 6 and 7). In many outgrowths it is not possible to distinguish cell boundaries, but in instances where they are evident, it is apparent that the shifting movements of the nuclei are but a rotation of the cells that contain them (Scammon, '15). The rotation processes are confined chiefly to the cells at the lower two-thirds of the glandular outgrowth. The fundic portion of the gland takes on a flask-like form as a direct result of these rotation processes (figs. 6 and 7).

8. With the completion of rotation, the cells at the fundic portion of the gland are placed, with reference to their long axes, at right angles to the lumen of the gland (fig. 7).

9. The fully differentiated gastric gland of *Squalus acanthias* is an unbranched, flask-shaped structure. The differentiation of the two cell-types does not occur.

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PLATES

PLATE 1

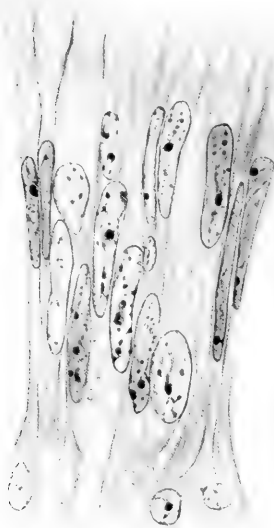
EXPLANATION OF FIGURES

The figures are all taken from the gastric epithelium of embryos fixed in a mixture of 10 per cent formalin and 2 per cent chromic acid.

2 The earliest recognizable stage in gland development of an embryo 133 mm. long. Iron haematoxylin-orange G stain. $\times 1000$.

3 The formation of definite gland rudiments in the epithelium of a specimen 137 mm. in length. Iron haematoxylin-orange G stain. $\times 1000$.

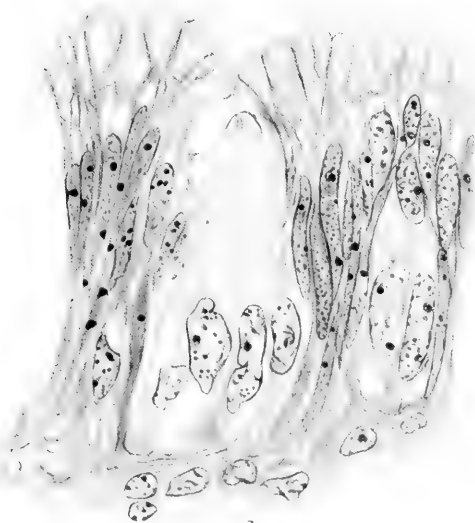
4 Outgrowth of a gland rudiment and extension into the underlying mesodermic tissue in an embryo 137 mm. long. Iron haematoxylin-orange G stain. $\times 1000$.



2



4



3

PLATE 2

EXPLANATION OF FIGURES

5 The glandular outgrowth has assumed a tubular form in a specimen 137 mm. in length. Iron haematoxylin-orange G. $\times 900$.

6 Shows the change in form of a glandular outgrowth as based on a rotation of cells in an embryo 137 mm. in length. Iron haematoxylin-orange G. $\times 900$.



PLATE 3

EXPLANATION OF FIGURE

7 The fully differentiated gastric gland of a specimen 146 mm. long. Iron haematoxylin-erythrosin. \times 1170.



Resumido por el autor, Gilman A. Drew.

Actividades sexuales del calamar, *Loligo pealii* (Les.)

II. El espermátforo: su estructura, eyaculación y formación.

Un espermátforo está compuesto de una masa de espermatozoides, una masa de material de cemento, un grupo de membranas que forman en conjunto un aparato eyaculador y vainas para cubrir la masa de esperma, y tónicas envolventes que suministran fuerza para la eyaculación. Durante esta última la masa de esperma y el cemento son expulsadas por un tubo que se evagina en el extremo oral del espermátforo. La masa de esperma se rodea de ciertas membranas que forman el reservorio espermático y se fija sobre la hembra por una masa de cemento que se acumula en el extremo del reservorio espermático. El esperma sale por un orificio situado en el extremo libre del reservorio. Los espermátforos se forman en el órgano espermátforico, porción especializada del espermoducto. Este órgano presenta un cierto número de divisiones, cada una de las cuales forma una porción del espermátforo. La cantidad de esperma suficiente para cada uno de estos entra en el órgano de una vez. Por acción ciliar este filamento de esperma se arrolla en una masa espiral cilíndrica y apretada que prosigue girando sobre su eje longitudinal durante su paso por el órgano. A esta estructura se suman materiales producidos en ciertas partes del órgano y de este modo se arrollan membranas y tónicas alrededor del espermátforo en vías de formación. Después que este se ha formado por completo, la túnica externa se contrae produciendo la turgescencia de dicha estructura. Los espermátforos una vez formados se acumulan en el saco espermátforico, en donde permanecen hasta que son utilizados.

Translation by José F. Nonidez
Columbia University

SEXUAL ACTIVITIES OF THE SQUID *LOLIGO PEALII* (LES.)

II. THE SPERMATOPHORE; ITS STRUCTURE, EJACULATION AND FORMATION

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FORTY-ONE FIGURES (SIX PLATES)

The spermatophores of Cephalopods have been mentioned in zoological literature by many writers, but most of the accounts are short, incomplete, and inaccurate, so there seems to be no real need for reviewing the literature as a whole.

By far the best account of the structure and ejaculation of spermatophores of Cephalopods I have seen is given by Émile-G. Racovitza, for *Rossia macrosoma* ('94) in a paper dealing with the habits and reproduction of the species. His descriptions and figures of the spermatophores are much more complete and accurate than those of earlier writers and there seems to be nothing added by later writers of very great importance.

To follow the method by which so complicated a structure is formed by added secretions on the inside of a duct, it is necessary to have rather elaborate figures representing the structure and ejaculation of the spermatophores, so it has seemed best to consider the whole question of structure, ejaculation, and formation together. A previous paper (Drew '11) on the sexual activities of the squid deals with the copulation, egg-laying, and fertilization, and might well follow the observations given in this paper as it deals with the use made of the completed spermatophores.

Part of the laboratory work, which forms the basis of this paper, was done at the University of Maine while I was connected

with the Biological Department of that university, and I am grateful for working space furnished me by the University of Arizona during the winter of 1917. By far the greater part of the work has been done at the Marine Biological Laboratory at Woods Hole, Massachusetts, and all of the material has been obtained at that station.

This species of squid is very abundant in the vicinity of Woods Hole, and any mature male taken from early in the spring until as late as September, and frequently later than that, is sure to have an abundance of spermatophores in the spermatophoric sac.

The spermatophores vary in size according to the size of the animal from which they are taken. Those from very small animals may not be over 8 mm. in length and those from large animals may be as much as 16 mm. in length. They are of course all similar in structure, but the small ones are softer and are not so easily handled in ejaculation observations as the larger ones. As might be expected, there are some slight individual variations in shape and size of parts in the spermatophores of different individuals. The spermatophores of each individual are practically identical in shape and appearance, but may vary slightly in size.

The number of spermatophores carried by each individual varies with the size of the animals (the smaller having fewer than the larger), with the season of the year, and with the frequency of copulation. May and June are probably the months when sexual activity is at its greatest. Usually at this time the spermatophoric sacs are gorged with spermatophores. A large individual may have as many as four hundred fully formed spermatophores stored at one time.

The formation of the spermatophores is evidently rather rapid. Several, perhaps several dozen, may be formed in a day. It is difficult to determine with anything like accuracy what the rate may be, but after a male has used large numbers of spermatophores in repeated copulations, a day or two is sufficient to bring the supply almost if not quite to normal.

STRUCTURE

Each spermatophore consists of a white opaque mass of spermatozoa surrounded by almost transparent liquids and membranes, a mass of material, rather opaque but not so opaque as the mass of spermatozoa, which lies against one end of it, and a brownish spiral filament together with a number of membranes of varying degrees of transparency on the other side of the body just mentioned. For convenience we may speak of these main divisions as the sperm mass (fig. 1, *SM*), the cement body (*CB*) which lies at one end of the sperm mass and was called by Racovitza the 'faux boyaux,' and the ejaculatory apparatus (*EA*), composed of the complicated group of membranes and the spiral filament which joins the cement body and occupies the smaller end of the spermatophore. It is also convenient to speak of the portion of the spermatophore occupied by the ejaculatory apparatus as the oral end, since from this end ejaculation takes place.

Figure 1, a complete spermatophore, shows that the aboral is considerably larger than the oral end. The sperm mass is little more than half the diameter of the spermatophore and does not extend to the extreme aboral end of the spermatophore. There is some difference in the amount of space posterior to the sperm mass, and likewise in the diameter of the portion of the spermatophore in which the sperm mass lies in different spermatophores. This is due largely to the fact that the spermatophores, when liberated take up water rapidly, and consequently swell and change in shape. This condition is easily controlled by passing the spermatophores into solutions of formaldehyde. Full strength formaldehyde will do no harm, but 10 per cent formalin is sufficient for ordinary purposes.

In studying the structure I have found that staining the formalin-treated spermatophores in dilute solutions of Ehrlich's triple stain or in Ehrlich's triacid stain and mounting in glycerin jelly has been very helpful. The stains are selective and stain different parts different colors or shades. The glycerin jelly will clear and preserve without shrinking the specimens badly.

The stains are not permanent, but when kept in the dark will last for some months. Formalin will harden gelatin, so mounts of formalin specimens become quite permanent, unless they are kept in a very damp place without being sealed.

The spermatophore is very turgid and elastic. This is due to the outer covering, the outer tunic (fig. 2, *OT*), which is a tough and elastic membrane. It is transparent, rather thin, and of about even thickness except at the extreme anterior end, where it becomes thickened and sculptured for the attachment of membranes of the ejaculatory apparatus, and where it is modified to form the cap (*C*) covering the oral end. The cap ultimately loosens and allows the spermatophore to ejaculate. The name cap which is applied to the portion that covers the oral end is somewhat confusing. The covering is formed by winding and cementing down around this end a thin leaf of outer tunic material which is continued as a long thin thread, the cap thread (*CT*), from the oral end of the spermatophore. This thread serves to loosen the winding of the cap when it is pulled, and ejaculation processes are immediately started.

The outer tunic gives the strength and elasticity to the spermatophore. The very great turgidity is due to the strain to which this tunic is subjected. When this tunic is punctured or cut, the contents escape rapidly and the tunic shrinks because of its elasticity.

Inside the outer tunic is the middle tunic (figs. 1 and 2, *MT*). This is closely applied to the outer tunic, thickened over the whole aboral end and gradually thins out orally, after the sperm mass is passed. The aboral portion is rather thick and granular, but transparent. After the region of the cement body is reached, the granular character is lost, and it is very difficult to determine whether a membrane is present or whether the space is filled with liquid. However, it can sometimes be easily traced. Under certain conditions of distension or ejaculation of the spermatophore, a line appears that can be accounted for only as the inner border of such a membrane. This portion is quite transparent, differing greatly in appearance from the granular membrane in the aboral end, but under favorable conditions

it may be traced to the oral end of the spermatophore, where it seems to end against a ridge on the inside of the outer tunic. The middle membrane of the ejaculatory apparatus, to be described later, is firmly attached to the other side of this same ridge. The middle tunic is soft, evidently elastic, forming an elastic cushion, and is evidently capable of taking up water rapidly. When a spermatophore is removed from a spermatophoric sac and placed in sea-water, the middle tunic immediately begins to increase in thickness and soon the spermatophore begins to ejaculate, or, if the cap holds, the outer tunic is ruptured by the increased internal pressure.

If the outer tunic be cut in such a spermatophore, the sperm mass is driven through the opening and the middle tunic thickens to occupy the space formerly occupied by the sperm mass. The combined swelling of the middle tunic and the elastic shrinking of the outer tunic nearly obliterate the space formerly occupied by the sperm mass. Evidently these two tunics are concerned in supplying the force that causes the ejaculation of the spermatophore.

Closely applied to the sperm mass is a very thin and not very definite membrane, the inner tunic (fig. 2, *IT*). This is frequently hard to identify, as it has nearly the same appearance as the mucilaginous material with which the sperm are mixed, and it is very closely applied to the mass. In the figures the part of the inner tunic covering the sperm mass is represented by a single line.

During the formation of the spermatophore, when the material of this tunic is wrapped around the sperm mass, it is easily distinguished in sections. In spermatophores which have not been completely formed, before final shrinking takes place, the coiling of the sperm thread of the sperm mass is quite distinct, and here the inner tunic is seen in the spaces between the coils of the sperm thread, separated slightly from the sperm mass.

Connecting the sperm mass and the cement body is a thin cylinder of transparent material, the connecting cylinder (fig. 6, *CC*) which seems to be a continuation of the core of the mucilaginous material with which the spermatozoa are mixed. The

inner tunic is seen here as a thin sheet surrounding this cylinder and extending from the sperm mass to the cement body.

On the cement body the inner tunic becomes applied to the outer membrane (fig. 2, *OM*) which covers this body. From this point on the inner tunic (*IT*) is thicker and very easily seen. It, together with the outer membrane, to which it is closely applied, leaves the cement body where it abruptly narrows and continues toward the oral end of the spermatophore nearly to its extremity, as part of the ejaculatory apparatus. The two structures are very similar in appearance, but the dividing line between them is distinct.

Just before reaching the point where the ejaculatory apparatus is thrown into loops, both the inner tunic and the outer membrane thicken to form a distinct ring. The dividing line between the two structures is easily followed for some distance into the thickening and is then hard to trace. I find, however, in the forming spermatophore, and occasionally in completely formed specimens which have been mounted some months in glycerin jelly, that the line of separation can be indistinctly traced nearly through this thickening, and I am inclined to think that the oral end of this thickening may be taken to be the extremity of the inner tunic. Beyond the thickening there is no indication of a double character and this part is probably the continuation of the outer membrane only. In the thickening the two structures are evidently more closely applied than elsewhere if, indeed, they are not fused.

The free end of the outer membrane is further on among the loops of the ejaculatory apparatus. It is easily seen in a specimen which is cut so that the loops of the ejaculatory apparatus straighten out, (fig. 3). This free ending of the inner tunic and outer membrane on the ejaculatory apparatus is of importance in studying the method of ejaculation.

The middle and inner tunics are entirely separate from one another. Where they touch they do not adhere. Between them is an actual or potential space (figs. 1 and 2, *SL*) filled with clear liquid. This space is always visible behind the sperm mass, in the region of the cement body, and along the ejacu-

latory apparatus. It is most easily seen behind the sperm mass, but there is evidently considerable liquid along the sides of the ejaculatory apparatus. This is especially shown during ejaculation.

The sperm mass (figs. 2 and 23 A, *SM*) is really composed of a thread or sheet of spermatozoa spirally coiled around a core of mucilaginous secretion, with the secretion extending between the loops of the sperm thread to the surface. The sperm forms a sort of elongated thin plate, coiled edgewise so one edge rests against the core and the other comes to the surface. The plate does not meet the surface and core at right angles, but is tipped slightly so the surface edge lags behind the core edge. By means of the secretion the plate composed of individual spermatozoa is consolidated into a very flexible cylindrical rod that has the character of a unit body. The secretion is tenacious and penetrates in between the spermatozoa, but the core and secretion between the loops of the sperm thread have very few spermatozoa embedded. Because of the appearance the sperm mass thus formed is frequently called the sperm rope. In the squid the loops of the spiral in the completed spermatophore are not very easily seen and, as a rope does not consist of a single strand coiled in this way, the term has no significance and may be misleading. The secretion mixes freely with sea-water, so the spermatozoa obtain their individual liberty promptly when the time comes for them to perform their function.

Just oral to the sperm mass is the cement body. This is more or less definitely attached to the sperm mass by the core of mucilaginous material, which extends orally from the sperm mass as a definite cylindrical filament, and by the inner tunic which continues from one to the other. The connection between the two structures is slight, but sufficient to connect them definitely together.

The cement body (fig. 2, *CB*) the function of which was not determined by Racovitza and was called by him the 'faux boyaux'—is a somewhat elongated pear-shaped body with the base somewhat less in diameter than that of the sperm mass

and the narrower elongated oral portion in contact with the end of the conspicuous spiral filament of the ejaculatory apparatus (figs. 2 and 23 A *CB*).

In the forming spermatophore the narrow end of the cement body, the hyaline core, (*HC*) is continued to the oral end of the spermatophore as a narrow cylindrical rod, which stains about the same as the material of the cement body. This fills the space inside the loops of the spiral filament and, oral to the spiral filament, the continuation of the lumen of the ejaculatory apparatus. I take it from Racovitza's figures and descriptions that this, which he calls the hyaline core, is persistent in the fully formed spermatophore of *Rossia*. In *Loligo* it is transient, disappearing, evidently by liquification, almost simultaneously with the completion of the formation of the spermatophore. The space it occupied remains as the lumen of the ejaculatory apparatus, which is probably filled with the liquid in the completed spermatophore.

In spermatophores in process of formation and frequently in freshly stained fully formed specimens, the cement material can be seen to be spirally wound. There is a central core, evidently a continuation of the cylinder extending between the sperm mass and the cement body, which is continued as a narrow cord the whole length of the hyaline core which extends orally from the cement body (figs. 23 and 23 A). In fully formed specimens the cement material usually does not show the spiral character plainly and the inner core may not be visible.

The cement body is probably covered entirely by the inner membrane, but over the large aboral end, where it is likewise covered by the outer membrane and the inner tunic, and where these membranes are fused tightly together, it cannot be traced as a separate membrane.

Over the narrow oral end of the cement body it is visible as a thin membrane which continues orally after leaving the cement body over the outer surface of the spiral filament. Still further orally, where there is no spiral filament, this membrane lies next to the lumen of the ejaculatory apparatus (fig. 2, *IM*). Formerly it was in contact at this point with the hyaline core.

The spiral filament (fig. 2, *SF*), while formed as a separate structure, evidently sticks to the inner membrane which covers it. The filament is brown, more or less granular, and is not of equal size and shape throughout. It is heaviest, with the coils most open, midway in its course, with both ends rather crowded. The loops of the spiral on the end next the cement body are very closely crowded, flattened, and the extreme end sometimes has the continuity of the thread broken so it is made up of consecutive fragments. The loops of the oral end of the filament become closely crowded, then more open and finally fade away so gradually that it is hard to determine where the filament ends. The function of the filament is not easily determined. It is evidently not a coiled spring and it seems to have very little elastic value. It seems probable that its chief function is to hold the lumen of the ejaculatory apparatus freely open so that evagination, to be described later, can be accomplished without tearing the membranes concerned. The rapidity of the ejaculation must be slowed somewhat to allow time for the oncoming cement body and sperm mass. The resistance caused by breaking the spiral filament into small fragments probably accomplishes this purpose.

Between the inner and the outer membranes is the middle membrane (fig. 2, *MM*). This is very transparent and frequently shows longitudinal striations, indicating the position of the successive windings of the sheet of which it is composed. It is much thicker than the other membranes and, while capable of much stretching, is evidently tough. It extends from the point where the outer membrane, together with the inner tunic, leaves the cement body, to the oral end of the spermatophore. At the aboral end, the tube formed by this membrane is closed by the oral end of the cement body. At the oral end this tube, which was open in formation (fig. 23, *MM*), is closed and closely applied to the inside of the cap where it spreads out laterally and is fastened by its lateral margin to the ridge of the outer tunic (fig. 2, *MM*). In this spreading and flattening process the lumen of the tube is also pressed out laterally so that in form the end is something like a pressed-in hollow rubber ball with

the tube formed by the middle membrane extending back from the concave side of the ball.

The term 'ejaculatory apparatus' has been applied to the inner membrane, together with the spiral filament, the middle membrane, and the outer membrane and inner tunic to their junction with the cement body. This is not a very satisfactory term as the spermatophore acts as a unit in ejaculation. That is, there is no one part that is active while the remainder are passive. The ejaculatory apparatus could not possibly deliver the sperm mass in position were it not for the elastic outer and middle tunics and their relations to liquids and structures. The term may, however, stand for want of a better one, since this portion is mostly concerned in ejaculation.

It may aid somewhat in understanding the arrangement of the parts of a spermatophore if we consider what is present in optical cross-sections through: 1, the region of the sperm mass; 2, the region of the aboral end of the cement body; 3, the region of the oral end of the cement body; 4, the region of the spiral filament, 5, the region just posterior to the cap. The parts cut will be mentioned in turn from the outside to the median axis (figs. 21 to 18).

1. The region of the sperm mass: 1, Outer tunic; 2, middle tunic; 3, space (actual or potential) filled with liquid; 4, inner tunic; 5, sperm mass.

2. The region of the aboral end of the cement body: 1, Outer tunic; 2, middle tunic; 3, space (usually actual) filled with liquid; 4, inner tunic; 5, outer membrane (if actually present fusee with the inner tunic), 6, inner membrane (probably); 7, cement body.

3. The region of the oral end of the cement body. 1, Outer tunic; 2, middle tunic; 3, space (usually actual) filled with liquid; 4, inner tunic; 5, outer membrane; 6, middle membrane; 7, inner membrane; 8, cement body.

4. The region of the spiral filament: 1, Outer tunic; 2, middle tunic; 3, space (actual) filled with liquid; 4, inner tunic; 5, outer membrane; 6, middle membrane; 7, inner membrane; 8, spiral filament; 9, lumen, probably filled with liquid, formerly filled with hyaline core.

5. The region just aboral to the cap: 1, Outer tunic; 2, middle tunic; 3, space (actual) filled with liquid; 4, middle membrane (the inner tunic and outer membranes do not extend this far); 5, inner membrane; 6, lumen (the spiral filament does not extend this far).

If each structure is considered in turn in their longitudinal relations we find:

1. The outer tunic is continuous over the whole spermatophore except at the oral end where there is a modification, the cap, which is spirally wound around the otherwise open tunic to form a closing mechanism. The cap has attached to it a long thread which, when pulled, serves to loosen the cap and thus liberate the enclosed mechanisms.

2. The middle tunic is continuous throughout the length of the spermatophore up to the thickened ridge on the outer tunic near the oral end, which it joins.

3. The inner tunic is continuous over the region of the sperm mass as a closely investing, thin membrane. It becomes thicker over the posterior end of the cement body. After leaving the cement body as an investing membrane it becomes a little thicker and closely covers the outer membrane. Near the region of the anterior extremity of the spiral filament this tunic ends with open mouth closely associated with the outer membrane.

4. The outer membrane probably begins at the aboral end of the cement body, but cannot be definitely distinguished from the inner tunic until near the place where both of these structures leave the cement body and, together, give the appearance of a double membrane. After leaving the cement body the outer membrane is applied to the middle membrane. A short distance orally from the end of the inner tunic the outer membrane also ends with an open mouth. It is important to understand that the oral portions of the inner tunic and the outer membrane together form a tube, closed at the aboral end where they are united to the cement body, and open at the oral end. The opening is of course closed by the other structures. There is, however, no organic union between these structures and the other membranes from the point where the outer membrane and

the inner tunic leave the cement body to invest the middle membrane.

5. The space, potential or actual, between the middle tunic on the outside, and the inner tunic, outer membrane and middle membrane on the inside, is continuous throughout the spermatophore. The liquid enclosed in this space serves the mechanical purpose of a lubricant and at the same time an easily flowing substance to which pressure is applied. The elastic force of the outer and middle tunics is transmitted through this liquid to the sperm mass and other structures during the act of ejaculation.

6. The sperm mass extends through the aboral two-thirds of the spermatophore, inside the inner tunic, which is closely applied and united to it.

7. The cement body is just oral to the sperm mass and attached to it by a connecting cylinder. The aboral end of the cement body is covered by the inner tunic, part of it at least by the outer membrane and possibly also by the inner membrane. If all are present, they are closely fused so they are hard to distinguish. The oral end of the cement body is covered by the inner membrane, outside of which come, in order, the middle membrane, outer membrane, and inner tunic. These are all easily distinguished from one another at this point.

8. The middle membrane forms a tube extending from the position where the inner tunic and the outer membrane leave the cement body to the oral end of the spermatophore. Just beneath the cap the oral end, which, although formed as an open tube, is now closed, becomes closely applied to the inside of the cap and is spread out laterally to the ridge on the outer tunic to which it is firmly attached. The open aboral end of the tube formed by the middle membrane is plugged by the small oral end of the cement body which is covered by the inner membrane. It has no organic connection with the outer membrane or with any part of the cement body, except for about one-third of the length of the surface that is applied to the inner membrane where it covers the cement body (fig. 23 A, PA). The portion next to the open mouth of the middle membrane ad-

heres to the inner membrane covering the cement body firmly, and in ejaculation is liberated only by the rupture of this membrane. It is important to understand that the open mouth of the middle membrane is directed aborally and the open mouths of the inner tunic and the outer membrane are directed orally. The one fits inside the other. The oral end of the middle membrane is a closed structure, like an indented hollow rubber ball, attached by its margin to the outer tunic and with the thin convex side (fig. 2, *MM*¹) applied to the under surface of the cap which closes the outer tunic. This part ruptures when the cap is loosened, so the lumen of the ejaculatory apparatus is opened to the outside (fig. 5, *MM*¹).

9. The inner membrane and spiral filament are united to the middle membrane. Their positions and relations are better shown by figures than by description. In ejaculation the inner membrane ruptures at the point where it joins the cement body (fig. 9, *PR*).

With these points in structure in mind we can now proceed with the method of ejaculation.

EJACULATION

In delivering the spermatophores the male grasps a bundle of them with the tip of the left ventral arm and quickly passes them into position (Drew, '11). The spermatophores leave the sexual duct of the male aboral ends first and the long threads connected with their caps, embedded in the secretions of the spermatophoric sac, drag behind. The sharp pull occasioned by the movement of the arm pulls on these threads and causes the caps to loosen. This starts the process of ejaculation. Under normal conditions the process is very rapid, occupying about ten seconds. This very rapid action of course makes it impossible to follow the ejaculation under a microscope even though the spermatophore is held in position and the thread pulled when all is ready. It was accordingly necessary to devise some method to slow down the movements.

Evidently the great tension of the elastic outer tunic has much to do with the process of ejaculation. Inasmuch as spermatophores

carefully removed from the spermatophoric sac without pulling the cap thread and placed in sea-water are very likely to ejaculate soon, and placed in fresh water will either ejaculate or burst very promptly, it is evident that osmotic action, in which the middle tunic is probably involved, plays an important part.

Elasticity and osmotic action accordingly have to be considered in searching for some method to slow down the action. It was found that formaldehyde affects probably both the elasticity and the osmotic properties, but that it evidently hardens the cement holding the cap, so it is very difficult to open it when needed, and that the various membranes of the spermatophore are soon so changed, possibly by hardening certain colloids, that ejaculation is not likely to be completed. These very properties were, however of the utmost value in the studies, for full-strength formaldehyde thrown on an ejaculating spermatophore will cause it to stop all action rather promptly. After some experience it became possible to allow the required amount of time to stop a spermatophore at the required stage of ejaculation by squirting full-strength formaldehyde on it just before it reaches the stage wanted. Such a spermatophore may then be stained in aqueous stains and mounted in glycerin jelly for study at leisure. It, of course, requires a great deal of time and patience to get some of the stages, but, as the figures showing the stages of ejaculation accompanying this paper are camera-lucida drawings of such specimens, it will be seen that it is possible to get the stages by this method.

Very many chemicals were tried to get the required slowing effects. Sugar solutions were good, but the membranes were soon weakened so the elasticity was destroyed. Magnesium chloride solutions have given the best results. The strength of the solution that works best seems to differ with spermatophores from different individuals, but a one-fourth saturated solution in sea-water has been very good.

The spermatophores are received from the spermatophoric sac directly into this solution, and in two or three minutes they will be ready for use. The turgidity is evidently effected, and where the spermatophores are left several hours, there may be

changes in elasticity and the freedom with which membranes will move on each other may be disturbed. After the spermatophores have been in the solution some minutes, if they are to be used for work for a long period, more sea-water may be used to dilute the solution. Generally it is best to use material that has recently been put into the solution.

The method used in studying ejaculation was to remove the spermatophore from the magnesium chloride solution to a watch-glass with a little sea-water, placed on a black background. The cap thread was then grasped with forceps and the whole spermatophore shaken. With a reasonably powerful engraver's glass held on the head with a spring the process of ejaculation may be watched, and with a large-mouthed pipette filled with formaldehyde the process can be stopped when desired. The time for ejaculation may be slowed down to take from a minute to two minutes, so it is possible to supplement observations made on the fixed material by observations on the ejaculating spermatophores.

It is necessary to concentrate attention on one portion at a time, but there is no difficulty in following movements of parts under the lens of a compound microscope. The chief trouble is in focusing attention on particular parts, for everything is moving at the same time and the mechanism is too complicated to be taken in at a glance and too large for all to be under a lens of sufficient power at one time.

As the process of ejaculation is somewhat complicated, a series of diagrams are given on plate 6, from which all portions not essential to understanding the process have been eliminated. By referring to these diagrams at this time it will be easier to follow the processes of ejaculation as they are given in other figures and descriptions.

The cap end of the cap thread is flattened and is apparently applied and cemented to the outer tunic in a somewhat spiral manner so the otherwise open end of the tunic is held shut. When the thread is pulled it loosens where cemented (fig. 4), and the end of the outer tunic is allowed to open. There is evidently some tearing, but not much.

With the opening of the tunic the portion of the middle membrane applied to the inside of the cap ruptures so that the lumen of the ejaculatory apparatus is opened to the outside of the spermatophore and the ejaculatory apparatus immediately begins to evaginate because of the pressure on the inside of the spermatophore (fig. 5). The evagination of that portion of the ejaculatory apparatus which is oral to the spiral filament is so rapid in the untreated spermatophore that the eye cannot follow it, but in the specimens treated with magnesium chloride it may be slowed down so the gradual evagination can be followed easily. As the ejaculatory apparatus evaginates, the diameter of the tube is greatly increased and the walls are correspondingly thinned.

There is a distinct pause in the evagination when the region of the spiral filament is reached (fig. 6). This is probably largely due to the stiffness of the filament itself, but may be influenced by the fact that other membranes are involved at about the same point.

Evidently the evagination of the first part of the ejaculatory apparatus is due to the pressure of the liquid between the middle and inner tunics that is in the oral end of the spermatophore. This is shown by the fact that the action is so rapid and by the further fact that the cement body and sperm mass are drawn away from each other (fig. 6). The sperm mass lags behind, so the connection between it and the cement body is stretched to its full extent.

After an instant's delay when the region of the spiral filament is reached, the tube continues to evaginate. The evagination here is continuous, but not nearly so rapid as the first part. The evaginated portion of the tube increases greatly in diameter, the walls become correspondingly thinner and the spiral filament is broken into minute fragments which continue to adhere to the outside of the evaginated tube, (fig. 7). As this process goes on, the free edge of the outer membrane adheres to what is now the inside of the evaginated middle membrane and is reflected so that this membrane, together with the inner tunic with which it is associated, is turned inside out (figs. 7 and 8, *OM* and *IT*).

Evidently the force that causes the evagination is still the elastic and osmotic force in the outer and middle tunics of the spermatophore acting through the liquid which fills the space between the walls of the evaginating tube.

The part played by the spiral filament seems to be largely, if not wholly, that of keeping the tube from collapsing with the pressure, but there may be some elastic force that aids in the evagination. The torsion that would be caused by the turning of a spiral spring might aid in the evagination, when once started, but there is no evidence that the filament is particularly elastic or partakes of the nature of a spring. The very fact that it is broken into minute fragments during the process of the evagination of the tube indicates that it can have no very great elastic properties, and probably indicates that it retards rather than accelerates the evagination of this part. It is necessary that evagination of this portion shall not be too rapid as the sperm mass must gain momentum and move along at a corresponding rate.

That such a filament may serve a very useful purpose in keeping the tube from collapsing or folding is evident. The freedom of the movements of the membranes concerned would be seriously interfered with if the tube were allowed to collapse or kink. The oral end of the tube does not need such a mechanism, as it is short and simple in construction and would naturally evaginate quickly with the pressure of the liquid between it and the outer wall. The same condition would not hold true for the much longer and more complicated tube that has the spiral filament.

It is of passing interest to note that there is a very general impression among zoologists who have no personal acquaintance with Cephalopod spermatophores that this spiral filament is really a spring, that it is used in discharging the sperm mass in the same mechanical way that a spring gun discharges its projectile, and that the discharge is through the end of the spermatophore farthest from the spring. There is, of course, no foundation of fact whatever for such an impression. It is simply arriving at conclusions from superficial appearances rather than by study and experimentation.

When evagination has proceeded as far as the oral end of the cement body, so that the end of this body begins to project through to the outside, the inner membrane is ruptured, so that this membrane, with the remnants of the spiral filament, is separated from the cement body (fig. 9, *PR*).

As evagination now proceeds, the oral end of the cement body projects into the sea-water. At this point ejaculation is retarded until the very great pressure behind the sperm mass forces this mass against the aboral end of the cement body, to which the inner surface of the middle membrane is attached, and so causes the middle portion of the cement body to be drawn out and around the sides of the aboral portion of the cement body in the form of a cap (fig. 11). The extreme oral end of the cement body, from which the inner membrane and spiral filament have been torn, appears as a knob or button on the otherwise smooth surface of the cement body.

It may be well, before proceeding with the other changes that are taking place, to call attention to the position of the oral portions of the inner tunic and outer membrane. In the evagination that has taken place the free ends of the inner tunic and outer membrane which originally enclosed the middle membrane have been turned back by the evaginating tube, so that the opening is directed toward the aboral end of the spermatophore, and, together, they form the inner lining of the evaginated tube as it now appears. In this process the sperm mass is being carried through the opening of the inner tunic and outer membrane and is being forced into the sac formed by them (figs. 9, 12, and 39).

As has already been pointed out, the inner tunic and outer membrane are firmly attached to the sides of the aboral end of the cement body so, when ejaculation has proceeded to this point, the membrane cannot be stripped further aborally. The part which has been turned back with the evaginating middle membrane thus forms a sac, with the cement body firmly attached to the closed end, and the pressure from behind forces the sperm mass into this sac.

The adhesion of the end of the middle membrane to the middle portion of the cement body not only serves to draw this cement body around the end of the sperm mass, but holds the sac in position to have the spermatozoa thoroughly and completely forced into it by pressure behind. It will be noticed that during this process the diameter of the aboral end of the spermatophore is greatly reduced, due to the elasticity of the outer tunic, and that the middle tunic swells, loses its granular appearance, and comes to occupy the space vacated by the sperm mass. At the same time the outer end of the evaginated ejaculatory apparatus becomes considerably expanded as the sperm mass is crowded into it (figs. 12, 13, and 14).

Continued pressure causes the walls of the cement body to burst (fig. 15). The end of the middle membrane is thus released and the sperm mass enclosed in the reflected inner tunic and outer membrane, smeared with cement from the ruptured cement body on its larger closed end, glides rapidly through the middle membrane and is free from all other mechanisms.

The covered sperm mass, which may be called a sperm reservoir (fig. 17), is usually somewhat coiled. The closed end is large and covered with cement, and the open end is small, with a thickened portion just behind the opening. The thickened portion seems to correspond to the thickened portion of the inner tunic and outer membrane, described in connection with the structure of the spermatophore, that lies a little aboral to the free end of this membrane. The thickened walls probably tend to prevent too rapid escape of the spermatozoa.

From the open mouth of this sperm reservoir of untreated specimens the spermatozoa escape in a constant cloud which reminds one of the smoke from an evenly discharging factory chimney. The discharge may go on for hours. When care is taken to provide an abundance of sea-water, such reservoirs will still be discharging twenty-four hours and more after they were liberated from the spermatophores.

Referring to the methods of copulation of the squid, given in a former paper (Drew, '11), it will be seen that when the spermatophores are carried to the mantle chamber of the female

they are held in position by the male long enough for them to discharge and to have the sperm reservoirs fixed by the cement on them to the tissue near the oviduct of the female. In this position each gives out its small cloud of sperm for some hours. If the female lays her eggs within the time they are active, insemination is assured. On the other hand, if the spermatophores are transferred to the region of the buccal membrane of the female, they are held in position by the male until they discharge and the sperm reservoirs are attached to the walls arranged for them. Here, as they discharge, the spermatozoa are directed, evidently by ciliary action, into the sperm receptacle where they are stored for future use.

The discharged empty case (fig. 16) is much smaller, especially in diameter, than it was before ejaculation. The outer tunic appears about as it did. The middle tunic is clear, not granular, and occupies most of the space inside the outer tunic. The evaginated tube that adheres to the oral end of the outer tunic is likewise less in diameter than it was at the time of evagination when there was pressure inside. The end of the tube attached to the outer tunic is clear and corresponds to the oral unornamented portion of the tube in the spermatophore. The region of the spiral filament is shown by the broken fragments adhering to the tube, and the outer end of this marked portion represents the end of the spiral filament and inner membrane that was attached to the oral end of the cement body. This is the point of rupture (*PR*). The remaining unornamented flaring tube is the part of the middle membrane which was in contact with the cement body. The outer third of this portion was firmly attached to the inner membrane that covered the cement body. By the breaking of this attachment the sperm reservoir, with the cement at the closed end, became free to be forced out of the case by the pressure behind it.

FORMATION

At first thought it is very difficult to understand how so complicated a structure as a spermatophore, with its numerous coats and structures, can be formed as a secretion inside the lumen of a glandular duct. To make the process clear it is necessary to know the structure of the duct in some detail.

The parts of the duct have received different names by different writers, and, inasmuch as the functions of the parts were not well understood at the time, the names that have been applied to them are generally not significant and should, I think, be abandoned as misleading.

A recent writer (Marchand, '07), who has covered this subject much more fully than has previously been done and who has made careful comparisons of the male ducts of a large number of Cephalopods, had analyzed the names previously given and made selections that suit his purpose, but as these names are applied without definite knowledge of the functions of the parts receiving the names and as more than one function is performed by a part to which he gives a single name, following the names he gives would seem to lead to even more confusion than to again change them.

The male genital organs of the squid are asymmetrical, only the testis and duct on the left side being present. The testis lies far posteriorly and dorsally (the terms posterior, anterior, dorsal, and ventral are used in the apparent rather than the true morphological sense). Just beyond the testis capsule the vas deferens shows a slight swelling, the ampulla of the vas deferens. From this point the vas deferens, at first a wavy and then a closely plaited tube, extends around the left side of the visceral mass to a point just posterior to the left branchial heart. Here the sexual duct enlarges to form a complicated, folded gland in which the spermatophores are formed.

The whole mass is frequently referred to as the spermatophoric gland. This is proper in the sense that the spermatophores are formed here, and it is not proper, inasmuch as it is not a single gland, but a series of glands and mechanical contrivances,

each portion of which has a definite individual function in the formation of spermatophores. For convenience we will call it the spermatophoric organ. This name will be applied to the whole structure, consisting of various glands and mechanisms, which extend from the vas deferens to the duct that carries the completed spermatophores to the spermatophoric (Needham's) sac. The spermatophoric organ is rather transparent, like most tissues of the squid. The forming spermatophores may be easily seen in the different parts of the organ. It is possible to cut the organ away from the visceral mass, place it in a watch-glass of sea-water and, under a compound microscope, see somewhat clearly the structures and positions of a forming spermatophore. By keeping the water changed on such an organ, its movements, which are very vigorous, will be kept up for nearly an hour and the forming spermatophores during the interval will move some distance. Within this organ the spermatophores are formed and completed.

The duct leading from the spermatophoric organ to the spermatophoric sac, which will be called the spermatophoric duct (frequently called the vas efferens, and by Marchand the distal vas deferens) carries the completed spermatophores for storage in the spermatophoric sac.

The vas deferens (figs. 29 and 30, *VD*) passes dorsal to the spermatophoric organ (between it and the general visceral mass) for about three-quarters of the length of the organ, where it joins the first of a series of structures that together form this organ (fig. 32, *VDO*).

The portion of the spermatophoric organ joined by the vas deferens I will call the mucilaginous gland (figs. 29 to 36). It secretes a sticky substance which is mixed with the spermatozoa and forms the material in which the sperm thread is imbedded, the cement body, and the hyaline core around which the spiral filament is wound. This gland is composed of two parts. One part (*MG*²) extends from the vas deferens to the next portion of the spermatophoric organ. This is referred to by Marchand as the second division of the spermatophoric gland (*vesicula seminalis*). Marchand uses the term spermatophoric gland

for three parts of what is here called the spermatophoric organ. The term does not serve my purpose, for the portion is more than a mere gland or indeed a series of glands. There are more parts to be described than the three divisions given by Marchand, and the term spermatophoric gland would indicate that the spermatophores are formed here, while they are only partly formed here.

The other portion of the mucilaginous gland (MG^1) forms a large outgrowth from the side of the portion just described. The opening of this portion is near the opening of the vas deferens and a considerable portion of the gland extends back between the viscera and the portion just described. This is called the first part of the spermatophoric gland by Marchand, and will be called the first part of the mucilaginous gland here.

In structure the two parts are much alike. Both have thick walls, thrown into folds on the inside. These folds are frequently joined by bridges, and in many places the deeper portions of the depressions between the ridges form pouches or sometimes tubules (Williams '08). The whole is, however, too open to form a true racemose or tubular gland. The cavity of each portion of the gland is extensive, forming a pelvis or basin in which the secretion is poured. The whole interior of the gland is ciliated, but the pelvis is particularly well supplied with cilia. The spermatozoa, entering from the vas deferens, pass into the pelvis of the second part of the mucilaginous gland (fig. 32), where they are mixed with secretion and the moving thread of sperm is covered with it. The spermatozoa do not enter part one of the mucilaginous gland, but are passed along a groove in part two past, but a little to one side of, the opening of part one. In the region of the groove, and for some distance along the side, especially along the side nearest part one, the cilia are large and numerous and serve to move the mixed sperm and secretion continuously toward and along this groove through this portion of the mucilaginous gland.

Possibly one-third of the distance from the vas deferens to the distal end of part two the walls of the groove are thrown into a few spiral ridges (fig. 34, *F*), between which the spermatozoa

are passed. The sperm thread is here flattened between the ridges and wound edgewise so one edge becomes the center and the other edge of the surface of the spirally wound sperm mass. As the spermatozoa are covered by the secretion from the gland, the secretion along that edge which forms the center becomes a continuous core in which there are few spermatozoa, and the secretion on the flat applied sides of the sperm thread stick the successive loops of the coil together. The sperm mass, as coiled, does not lie with flat applied surfaces of the loops at right angles to the central core, but the edge applied to the surface lags a little behind the edge at the central core. The surface edge is accordingly nearer the cement body than the core edge.

In longitudinal section the sperm mass thus appears like a series of small open funnels with the small ends directed toward the aboral end of the spermatophore. The spaces between the funnels, together with the core that would occupy the small open ends of the funnels is filled with sticky material furnished by the mucilaginous gland. From the location of the ridges which serve to coil the thread, through the remainder of the mucilaginous gland and through succeeding glands, the sperm mass is rotated on its longitudinal axis by the action of the cilia in the groove in which it lies. It is through this longitudinal rotation that the sperm mass, molded by the ridges between which it passes, is coiled into the form described. The sperm mass, coiled in this way, is usually called the sperm rope. It should be borne in mind that the coil consists of a single flattened strand and not a number of strands as is the case with a rope (fig. 23 A; *SM*).

Spermatozoa continue to issue from the vas deferens and the sperm mass continues to form until the end first formed reaches some distance past the limit of the mucilaginous gland to a point about opposite the notch (fig. 32, *C*¹). The sphincter muscle around the opening of the vas deferens then contracts and no more spermatozoa are allowed to enter. When the free spermatozoa are wound into the coil the charge of sperm for one spermatophore is complete and the sperm mass is in final form. ,

As completed the sperm mass is cylindrical, with slightly tapering ends. The surface is smooth, the coiled thread being visible, but the coils are not prominent. The free surface is covered by a small amount of the mucilaginous material. In staining, the spermatozoa take haematoxylin, or other nuclear stains, and the mucilaginous material eosin. Scattered spermatozoa are found in the mucilaginous material, but there are not many of them. In this condition the sperm mass appears much as it does in the completed spermatophore, except that the coils of the sperm thread are a little more open and more easily seen. The change is to be accounted for by the pressure applied in the completed spermatophore by the elastic outer tunic.

As the sperm mass passes back through the mucilaginous gland, the groove in which it lies is formed by an overhanging ridge, an arrangement that becomes very prominent in the succeeding part of the spermatophoric organ (fig. 36, *GR*).

As the sperm mass passes out of the mucilaginous gland the cement body is attached to the end which leaves the gland last. This body is evidently formed by the mucilaginous gland, but I have not observed the actual process of formation. It has been seen immediately after it has left this gland, and, as it must be formed before the coiled filament is laid down, and the coiled filament is formed just beyond the mucilaginous gland, there can be no alternative as to its place of formation.

So far, I have not been able to determine whether parts one and two of the mucilaginous gland have the same function. Possibly one of these portions is concerned in the formation of the cement body alone, but I have not been able to find evidence on the point. With various stains these glands appear alike and the secretion in the sperm mass and the material of the cement body have similar affinities for stains. There seems to be a difference in composition however, for the cement hardens so as to stick permanently to bodies in sea-water, while the mucilaginous material mingled with the sperm mixes freely with sea-water and liberates the spermatozoa. This difference in composition has led me to search diligently for the exact

place and method of formation of the cement body, but thus far I have not been successful.

As the sperm mass and cement body leave the mucilaginous gland and are passed along the spermatophoric organ, a thin thread of mucilaginous material is formed which is continuous with the cement body. This continues to be formed as the forming spermatophore passes on, and becomes the hyaline core (fig. 23 *HC*) around which the spiral filament is wound. Racovitza, ('94) calls this the hyaline core in his description of the spermatophore of *Rossia*, where it evidently persists in the fully formed spermatophore. In the squid it is present only during the formation of the spermatophore and disappears before the spermatophore becomes functional.

The part into which the sperm mass and cement body is passed from the mucilaginous gland is thick-walled and granular, but the inside is smooth, not thrown into ridges and grooves as in the mucilaginous gland, nor are there sacules or tubules in its structure. The inner surfaces are smooth and strongly ciliated. The upper surface of the wall (the surface toward the visceral mass) is thrown into a very prominent ridge (figs. 32 and 34, *GR*) very similar in appearance to the typhlosole in the intestine of an earthworm, except that it is not bilaterally symmetrical. One margin of the ridge is drawn to the side and overhangs to form a very definite ciliated groove (fig. 34, *G*), along which the forming spermatophore is passed, moved by the cilia and by movements of the organ, and kept constantly rotating on its longitudinal axis.

The general structure of this portion of the spermatophoric organ is essentially the same from the mucilaginous gland to the narrow duct near the anterior end of the organ, but at least two divisions may be recognized in it. Externally the boundaries of these divisions are roughly marked by constrictions, the first of which (figs. 29 and 32, *C*¹) may be taken as the boundary of the mucilaginous gland and the second (*C*²) the boundary between two functional parts which show very similar structure. Marchand refers to these two divisions jointly as the third part of the spermatophoric gland. As the two parts are functionally

quite different it will be convenient to refer to them by different names.

The first part (figs. 29 and 32, *EG*) is slightly swollen and in it are formed the membranes of the ejaculatory apparatus. I therefore call it the ejaculatory apparatus gland. It is true that one of the membranes, the inner tunic, continues over the sperm mass so this portion actually forms more than the ejaculatory apparatus, but this term answers very well. The remaining portion (figs. 29 and 32, *MTG*) forms the middle tunic and will be called the middle tunic gland.

The sperm mass and cement body enter the ejaculatory apparatus gland, with the hyaline core still being formed in the mucilaginous gland, and moves slowly through it, receiving the inner tunic at the distal end of this gland, near the notch which separates this gland from the middle tunic gland. The material secreted by the glandular walls of this portion of the organ is moved by the cilia over the edge of the ridge. The slowly rotating sperm mass thus has this material wound around it as a thin sheet. Parts of the gland between this point and the mucilaginous gland are at the same time secreting materials that are being wound into other parts. Bear in mind that after the cement body leaves the mucilaginous gland, the hyaline core continues to be secreted by it.

The ridge, under the edge of which the sperm mass and cement body have passed, has a groove across its convex surface at a point about opposite the notch marking the boundary between the mucilaginous and the ejaculatory apparatus glands. This groove (fig. 32, *SFG*) is not very deep, but is easily seen in dissections of spermatophoric organs which have been preserved in formalin. It extends diagonally from one side of the ridge to the other and, on the side where the forming spermatophore passes, is deep enough to join the groove in which it lies. Just after the cement body passes its end the material that forms the spiral filament passes along this diagonal groove and, because of the rotation of the forming spermatophore, is wound around the hyaline core.

Immediately beyond this groove the material for the inner membrane is secreted and wound on as a sheet. The inner

membrane thus covers the cement body and the outside of the spiral filament, to both of which it adheres firmly. Orally to the spiral filament, the inner membrane covers the hyaline core.

A little further on the middle membrane is formed. The sheet of which it is formed is thin, but is wound around many times in building up this comparatively thick membrane. What causes the aboral end to be so definitely limited has not been determined.

The portion of the gland immediately following that which forms the middle membrane forms the outer membrane and that which follows, as already stated, forms the inner tunic.

All of these structures (figs 23 and 23 A), the inner membrane (*IM*), middle membrane (*MM*), outer membrane (*OM*), and inner tunic (*IT*), are formed in the same manner and the gland in which they are formed shows no definite change in structure from one part to the other. Apparently all are being formed at the same time and the formation of each part stops when it is completed. There seems to be nothing visible that limits the extent of the formation of each structure.

Some membranes adhere to others with which they come in contact and some do not. Thus the inner membrane forms a covering for the cement and adheres to the spiral filament and middle membrane. The outer membrane adheres to the inner membrane over the cement body, where they come in contact, and to the inner tunic, but not to the middle membrane.

It is perhaps as well to call attention to certain peculiarities in forming structures here as anywhere. The core of mucilaginous material in the sperm mass seems to be continued forward into and through the cement body (fig. 23 A). The connecting cylinder between the two parts is very prominent. The cement is seen to be spirally wound around this core in the partially formed spermatophore and the core is continued on throughout the length of the hyaline core as a much smaller core. The hyaline core is evidently continuous with the cement material.

The inner core does not stain heavily with any of the stains and seems to be distinct in composition from the cement material.

It is much more like the mucilaginous material in the sperm mass, but it has not just the same staining properties.

How these parts are formed is not known. Possibly the mucilaginous substance binding the sperm mass is continuous as a core and the cement substance and hyaline core are similar substances wound around the central core. If this be the case the mucilaginous gland must consist of two functional parts.

A second point has to do with the spiral filament. This seems to lie directly against the hyaline core, with the inner membrane covering it. The space between the loops of the spiral filament which extends from the inner membrane to the hyaline core is evidently filled with some substance that never stains and is apparently liquid. The hyaline core never bulges much between the loops of the spiral filament, and the inner membrane is never pressed in much between these loops. With the pressure that is put upon the contents of the spermatophore when it is completed—by the elastic outer tunic, even before the hyaline core disappears—there would be distortions were there not a supporting liquid in this space.

It is not difficult to understand how each of the layers described are formed when we bear in mind that each is wound around the slowly rotating mass as it proceeds through the duct. The invisible part, the part connected with the nervous mechanism that sees to it that each secretion is started and stopped at the proper time to make the whole a complete, well-formed complicated machine, is not more remarkable than many other nervously controlled mechanisms.

The forming spermatophore has now passed well back into the middle tunic gland, and by the time the structures described have been completed the first formed end of the sperm mass lies near the distal end of this gland.

As previously stated, the structure of the middle tunic gland (figs. 29 to 36, *MTG*) is essentially the same as that of the ejaculatory apparatus gland. The middle tunic is formed by winding a sheet of secretion around the rotating mass as in the membrane described. There is a little liquid between the middle

and inner tunics so the two do not adhere at any place. The middle tunic is of about uniform thickness over the part occupied by the sperm mass. It is thinner and less granular from this point to the oral end (figs. 23 and 23A, *MT*).

The forming spermatophore apparently remains in this part of the organ for some time; the sperm mass, cement body and part of the ejaculatory apparatus lying in the middle tunic gland, and the forward part of the ejaculatory apparatus lying in the ejaculatory apparatus gland. At this time the forming spermatophore is very much larger than the completed structure. It is sticky and soft so that when it is removed from the organ it remains bent in any shape in which it is placed, provided the bends be not abrupt. Before it is completed and functional, the forward end becomes much folded so the length is greatly decreased and all is shrunk so it is much less in diameter. The shrinking must effect length as well as diameter. All these changes are associated with putting on the outer tunic.

Before leaving the middle tunic gland, mention should be made of a narrow tube that joins its distal end (figs. 31, 32, and 34, *X*). The lumen of this duct is lined with epithelium lying directly on connective tissue. The walls are not glandular and the epithelium, which is ciliated, is evidently not composed of actively secreting cells. I am unable to assign any function to this tube. It has been suggested, by Marchand, that it may represent a degenerated part of the originally paired sexual ducts, only the left of which is functional. I have no information that throws light on this subject, but the point of junction in the course of a highly modified section of the duct is not what might be expected if this were the case.

I have not been able to observe the actual formation of the outer tunic. In the specimens I have examined the outer tunic is never present while the forming spermatophore is in the middle tunic gland. The outer tunic is always present in a spermatophore that has reached the next large division, which, though I am not entirely sure of its function, I call the hardening gland (figs. 29 to 36, *HG*). Marchand speaks of this gland as the accessory gland (*prostata*), a term with no functional

meaning. It can in no way be compared with the prostate gland of vertebrates. The inner walls of the gland are marked by various connecting ridges which project into the large cavity of the gland. The gland forms a blind sac with only one duct.

Only the end of the spermatophore that contains the sperm mass is pushed back into the hardening gland, and, as has been said, when the spermatophore is pushed into this gland the outer tunic is always present.

The connecting duct between the middle tunic gland and the hardening gland is relatively small, but the walls are highly glandular. The duct forms a bent cylindrical tube with a lumen that corresponds pretty well with the diameter of the spermatophore. I have not succeeded in removing spermatophores passing through this portion without injuring them and in sections the injury of the spermatophores is usually considerable. I find, however, that the material of the outer tunic is present on parts of the spermatophore that have not reached the hardening gland, so this narrow gland must be responsible for its formation. It may therefore be called the outer tunic gland (figs. 29 to 33, *OTG*). Inasmuch as spermatophores are seldom found in this gland, they probably pass through it rather rapidly.

The end containing the sperm mass is passed back into the hardening gland to about the level of the cement body. The region of the cement body and ejaculatory apparatus never enter this gland. The aboral end of a spermatophore, when present in this gland, projects into its lumen from the narrow outer tunic gland without touching its walls. The spermatophore has definite outlines, the outer tunic is fully formed and not sticky, and the liquid in the lumen of the gland is transparent and not noticeably viscid. When the gland is opened in sea-water, the secretion that mixes with the water is visible only because it has a different refractive index. It mixes readily with the water and disappears. In sections of the organ the contents of this gland frequently show some coagulated and stained material which probably comes from the secretion.

While the aboral end of the spermatophore is in the hardening gland the oral end is passed along the outer tunic gland to

the position of the opening from the side of this gland. This opening communicates with a complicated portion in which the spermatophore is completed. Marchand calls this (appendix) the blind sac of the distal vas deferens. It is not a true blind sac, as it has two openings, and the term appendix, which has been applied by other writers, has no meaning. I will call this (figs. 29 to 32, *FG*) the finishing gland. In passing the oral end of the spermatophore from the outer tunic gland into the opening of the duct leading to the finishing gland (fig. 32, *FD*) this end of the spermatophore is considerably folded, and, as the further movement is now with this end directed forward, the folds are held and compressed while the outer tunic hardens around them.

Just how much of the gland is responsible for the formation of the outer tunic is uncertain, but judging from the structure of the gland, the appearance of the tunic as seen in sections, and the appearance of spermatophores removed from the gland, I am inclined to think that the whole structure, from the end of the middle tunic gland to the end which extends into the hardening gland, is very active and that the duct leading from this portion to the finishing gland, the finishing gland duct, and the finishing gland itself, adds to the outer tunic over the oral end of the spermatophore.

Spermatophores taken from this position in the organ exhibit great differences in the appearance of the oral ends, and, as spermatophores are common in this position, the meaning probably is that the spermatophore is held here until the oral end is shaped and covered. It is then passed, oral end first, down the duct to the pointed end of the finishing gland.

The duct to the finishing gland is much larger than the lumen of the outer tunic gland and has a very definite groove along the side away from the visceral mass, which ends on the side of the finishing gland in a pouch (figs. 29 to 32, *PF*). The spermatophore usually lies in the part of the duct away from this groove and pouch, but in a few cases I have found the oral end of the spermatophore in this pouch. This position may not have been normal, for, in opened animals, the mechanism con-

trolling the movements of the parts of the spermatophoric organ, and accordingly the forming spermatophores, must be badly interfered with.

In a spermatophore removed from this position the outer tunic over the aboral end is well formed and normal in appearance. That over the oral end is thin, somewhat opaque, and adheres to a needle. It is most difficult to get specimens at this stage of formation free without injury. Figure 24 shows the oral end of the only really perfect specimen I have been able to remove.

In passing into the finishing gland the oral end of the spermatophore is pressed forward into the pointed end of the gland and evidently receives further additions to the outer tunic. The aboral end of the spermatophore is now free from the hardening gland and the secretions from this gland are free to find their way into the finishing gland. Whether this actually takes place I do not know, but the oral end, which just before was covered by a thin, somewhat opaque and sticky outer tunic, changes in form and appearance to that of the completed spermatophore.

The last processes in the change have to do with the formation of the cap. As shrinkage takes place, the oral end of the ejaculatory apparatus becomes further coiled and the cap region is bulged outward by the end of the tube formed by the inner and middle membrane (fig. 25). The cap thickens and the tube in question is forced over and finally pressed out sideways so the lumen of the tube is spread to correspond to the shape of the cap (figs. 26 and 27). The margins of the tube that come in contact with the margins of the swollen cap are fastened to the ridge of the outer tunic along the borders of the cap, and further shrinkage brings the spermatophore into functional form.

It is while in the finishing gland that the spermatophore shrinks into the finished size and the outer tunic becomes normally turgid and elastic. Here the hyaline core disappears probably becoming liquid. When the spermatophore starts down the spermatophoric duct it is completed in form and capable of normal ejaculation. A slight shrinkage, especially in

the region of the cap (fig. 28) will take place, but otherwise all is completed.

I have not been able to determine just how the thread that extends free from the cap is formed. It was first seen shortly before the oral end of the spermatophore enters the spermatophoric duct. A small glandular tube (figs. 31 to 33, *Y*) lies along the spermatophoric duct, and opens into the finishing gland near where this gland opens into the spermatophoric duct. The lumen of this duct is flattened in cross-section and the position of its opening is so near the point where the thread is first seen that I have been inclined to the belief that secretions from this gland form the thread. I have, however, no real evidence.

The spermatophore passes down the spermatophoric duct and enters the spermatophoric sac oral end first, with the cap thread lying by its side. Here it reverses ends again as the spermatophoric sac extends posteriorly beyond the spermatophoric duct a distance equivalent to the length of a spermatophore.

Each successive spermatophore crowds its predecessor sideways and by forcing its oral end into the posterior pointed end of the spermatophoric sac causes the preceding spermatophore to move, aboral end forward, further into the spermatophore sac. Successive spermatophores are thus arranged in a spiral manner inside the sac, and the cap threads trail back from their oral ends. The last spermatophore to enter the sac has its oral end slightly posterior to the oral end of the spermatophore that preceded it into the sac.

The walls of the spermatophoric sac are muscular, and spiral lamellae, extending into its interior (fig. 33 to 36, *SS*), keep the spermatophores in position, practically parallel to each other, but spirally arranged, with the aboral ends moving forward. The muscular action of the spermatophoric sac is evidently responsible for the most of the movements of the spermatophores it contains.

Where the spermatophoric sac joins the outer muscular duct, commonly called the penis (a term somewhat misleading as to function), the spermatophores largely lose their spiral arrange-

ment and become arranged in groups of from twenty to forty or more, parallel to each other and filling the lumen of the duct. Thus, when they are ejected from the penis and grasped by the hectocotylized arm, an even group, with their aboral ends forward and the threads still embedded in the secretion of the penis, is presented to the grasping arm.

SUMMARY

Spermatophore structure. The contents of the spermatophore are referred to as the sperm mass, cement body, and ejaculatory apparatus.

The sperm mass consists of the spermatozoa surrounded by and mixed with a mucilaginous material which mixes readily with water. It is the proper delivery of the sperm mass that is the essential action of the spermatophore.

The cement body contains the sticky material that finally sticks the reservoir, into which the sperm mass is forced, in position on the female.

The ejaculatory apparatus consists of membranous tubes and structures that together form by their evagination, the conducting tube through which the cement body and sperm mass are forced, and the sperm reservoir into which the sperm mass is forced.

The contents of the spermatophore, as described, are enclosed inside a very elastic outer tunic and a middle tunic that is elastic and capable of taking up water rapidly. Together these tunics supply the power necessary for ejaculation of the spermatophore.

The outer tunic is closed by a cap which is cemented in position and may be loosened by pulling the thread connected with the cap.

Spermatophore ejaculation. When the cap loosens the force supplied by the elastic outer tunic and osmotic middle tunic causes the ejaculatory apparatus to evaginate. In doing so the two outer coats of this apparatus, the inner tunic and the outer membrane, are reflected to form the sperm reservoir into which the sperm mass is forced. The continued action of the outer

and inner tunics forces the reservoir containing the sperm mass out, ruptures the cement body and smears the cement over the closed end of the sperm reservoir.

In this condition this body is freed from the remainder of the spermatophore and is normally stuck in position on the female by the cement.

Reference to the diagrams on plate 6 will aid in understanding the essential processes of ejaculation.

Spermatophore formation. The spermatophore is completely formed inside of that portion of the sexual duct called the spermatophoric organ. This is a complicated series of continuous glands, in the lumens of which the forming mass is kept rotating on its longitudinal axis. By this rotation the sperm mass, cement body, spiral filament, and the various enclosing membranes are spirally twisted and wrapped into position as the mass moves along the lumen of the organ.

When fully formed, the whole spermatophore undergoes a shrinking process by which the elastic outer membrane is left in a state of high tension which makes the whole spermatophore turgid and ready to ejaculate.

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EXPLANATION OF FIGURES

With the exception of figures 32 and 37 to 41, all of the figures were outlined with the aid of a camera lucida. Where cut surfaces are shown on spermatophores and spermatophoric organs the relations were worked out by study and shown for convenience of those interested in the paper. It is hardly necessary to say these were added after the camera-lucida sketches were made.

The sizes of spermatophores and spermatophoric organs differ with the sizes of the individuals from which they were obtained. This explains the differences in the size of the figures. The spermatophores were all drawn with the same magnification except figures 1, 12, 16, and 17, which are not so highly magnified. The sections of the spermatophores shown in figures 18 and 22 were considerably broken in preparation. While their outlines were obtained with the aid of a camera lucida, the damage was repaired by study. The figures are not from sections of the same spermatophore.

Large spermatophores measure 16 mm. in length, but 13 mm. is more common. During formation they are larger, but they shrink to their final size after they are otherwise fully formed.

ABBREVIATIONS

- C*, cap covering the oral end of the spermatophore.
C¹, constriction separating the mucilaginous gland from the ejaculatory apparatus gland.
C², constriction separating the ejaculatory apparatus gland from the middle tunic gland.
CB, cement body.
CB¹, cement liberated by rupture of cement body.
CC, connecting cylinder between the sperm mass and the cement body. In forming specimens the connecting cylinder is continuous with material which extends the length of the sperm mass, cement body, and hyaline core. In fully formed specimens this material may sometimes be distinguished in places.
CT, cap thread. When this is pulled the cap is normally loosened and the spermatophore ejaculates.
EA, ejaculatory apparatus. This term is slightly misleading, as the process of ejaculation is not confined to this part.
EG, ejaculatory apparatus gland. This term is not quite accurate, as other portions than the so-called ejaculatory apparatus are formed by this gland.
F, folds that serve to wind the sperm thread into a spiral. In the position shown in figure 34 they are much smaller than they are a little further along in the groove.
FD, finishing gland duct, connecting the finishing gland with the outer tunic gland.
FG, finishing gland. Where the cap and cap thread are formed and where the shrinking of the spermatophores is completed.
G, groove along which forming spermatophores pass. In some figures the forming spermatophores are present.

- GR*, gland ridge; typhlosole-like in appearance. Under one edge of this ridge is the groove along which the forming spermatophores are passed.
- HC*, hyaline core. Present in forming spermatophores, but later disappears, probably by liquefaction, possibly by withdrawal to the cement body.
- HG*, hardening gland. This may not be properly named. Only the aboral end of the spermatophore is thrust into this gland. In this position the aboral end of the spermatophore is always covered by the outer tunic, which is smooth elastic, and not sticky. The hardening of the oral end of the spermatophore takes place in the finishing gland, possibly by secretions delivered with the spermatophore from the hardening gland, possibly by secretions furnished by the finishing gland itself.
- IM*, inner membrane. A membrane of the ejaculatory apparatus and a covering for at least a portion of the cement body. On its inner surface it bears the spiral filament. It is so thin it has been represented by a line.
- IT*, inner tunic. Inconspicuous and represented by a line over the sperm mass and connecting cylinder, becoming thicker and more conspicuous over the ejaculatory apparatus, where, with the outer membrane, a double membrane is formed. This becomes part of the covering of the sperm reservoir when this is discharged from the spermatophore.
- MM*, middle membrane. A conspicuous membrane of the ejaculatory apparatus. The tube formed by it is firmly attached to the outer tunic at the oral end and has its open mouth applied to the shoulder of the cement body beneath the outer membrane.
- MM¹*, middle membrane, cap end. This portion ruptures when ejaculation of the spermatophore begins.
- MT*, middle tunic. Probably of a highly osmotic material that furnishes part of the power which causes ejaculation of the spermatophore.
- MTG*, middle tunic gland.
- MG¹*, mucilaginous gland, part one.
- MG²*, mucilaginous gland, part two. The separate functions of these two parts have not been determined, but together they form the secretions with which the spermatozoa are mixed, and which form the connecting cylinder, the hyaline core, and the cement body.
- OM*, outer membrane. A portion of the ejaculatory apparatus. For most of its length it is intimately associated with the inner tunic so the two appear as a double membrane. The tube which it forms is applied to the middle membrane and ends with a free opening near the oral end of the spermatophore. With the inner tunic it forms the sperm reservoir.
- OT*, outer tunic. A highly elastic tough outer covering. This, together with the middle tunic, furnishes the power that causes ejaculation. When the spermatophore nears completion this tunic shrinks until it is under great tension and the spermatophore becomes very turgid as the result.
- OTG*, outer tunic gland. It is possible this may not be responsible for the formation of the outer tunic, but it probably is.
- PA*, point where adhesion between the middle and inner membranes covering the cement body becomes strong. From this point to the end of the middle membrane they adhere firmly. As the spermatophore nears completion, the point of adhesion is not so easily seen, but during ejaculation the adhesion is seen to be strong.

- PF*, pouch on the finishing gland, of unknown function. This pouch is connected with the lumen of the finishing gland duct and with the finishing gland itself as a sort of diverticulum.
- PR*, point of rupture of the inner membrane. During ejaculation the inner membrane and the spiral filament separate from the oral end of the cement body, As ejaculation proceeds they form the outer covering of the tube through which the sperm mass is forced. The extreme outer end of this tube is free from them as the middle membrane, which forms the extreme outer end, extends along the cement body past the point of rupture.
- S*, 18, 19, 20, 21, 22, lines on figure 1 and figure 16 indicating the planes of sections of spermatophores represented by figures bearing the same numbers.
- S*, 33, 34, 35, 36, lines on figure 29 that indicate the planes of sections of the spermatophoric organ represented by figures bearing the same numbers.
- SD*, spermatophoric duct, connecting the finishing gland with the spermatophoric sac. When a spermatophore starts into this duct from the finishing gland it is completely formed, except that a slight shrinking, especially in the region of the oral end, will still take place. By the time the spermatophoric sac is reached the shrinking is complete.
- SF*, spiral filament. This is fastened to the inner membrane and seems to serve to keeping lumen of the ejaculatory apparatus open. The material of which it is composed is brittle and the filament is broken into small fragments as the tubes composing the ejaculatory apparatus are everted. The same letters have been used for the filament while the coils are distinct and for the broken fragments that remain sticking to the outside of the evaginated inner membrane. See figure 7.
- SFG*, spiral filament groove. The material from which the filament is formed is passed along this groove to the forming spermatophore which is passing along the groove underneath the ridge across which the spiral filament groove cuts.
- SL*, space filled with liquid. This liquid originates in the middle tunic gland. It does not stain and evidently has only the double purpose of lubrication and transmission of pressure.
- SM*, sperm mass. In lettering the same letters have been used for the mass of spermatozoa, whether in position in the spermatophore, during ejaculation, or in the sperm reservoir after ejaculation is complete. It should be borne in mind that the arrangement is changed so the original sperm mass is disorganized entirely by the time it reaches the sperm reservoir. As the disorganization is a continuous process in ejaculation, it seems more confusing to attempt to designate it by different letters and names than to use the same letters with this explanation.
- SS*, spermatophoric sac. The receptacle in which the completed spermatophores are stored. Because of size it is shown only in the figures of cross-sections of the spermatophoric organ. It is really not a part of the spermatophoric organ, but a storage receptacle. It receives the spermatophores from the spermatophoric duct which comes from the finishing gland, and delivers them through the penis.
- VD*, vas deferens. This plaited tube joins the testis with the spermatophoric organ and delivers the completely formed spermatozoa to it.

VDO, vas deferens opening into the mucilaginous gland. The opening is provided with a sphincter muscle and the spermatozoa are allowed to enter only at definite intervals.

X, a duct of unknown function. A ciliated, not glandular, duct which opens into the distal extremity of the middle tunic gland. It has been suggested that this represents the vestige of the right vas deferens, but this seems rather doubtful.

Y, a glandular duct of unknown function that joins the finishing gland near the opening to the spermatophoric duct.

PLATE 1

EXPLANATION OF FIGURES

1 Spermatophore completely formed as taken from the spermatophoric sac. The cap thread is shown broken at a little less than one-half the normal length. At this magnification the outer tunic is represented by a single line and ejaculatory apparatus details are not shown. $\times 20$ diameters.

2 Oral end of a spermatophore. $\times 70$ diameters.

3 Oral end of a spermatophore. Represented as cut when fresh so the ejaculatory apparatus has expanded, uncoiled and thrust back through the cut ends of the outer and middle tunics. In this condition the free oral ends of the outer membrane, *OM*, and the inner tunic *IT*, are more easily seen. $\times 70$ diameters.

4 Oral end of a spermatophore. Shown with the cap thread loosening. $\times 70$ diameters.

5 Oral end of a spermatophore, after the cap has opened and the ejaculatory apparatus had begun to evaginate. $\times 70$ diameters.

6 Oral end of a spermatophore, after the oral unornamented portion of the ejaculatory apparatus has evaginated and before the portion bearing the spiral filament has begun to evaginate. There is a slight pause at this stage of ejaculation. $\times 70$ diameters.

7 Oral end of a spermatophore, when the portion of the ejaculatory apparatus bearing the spiral filament is evaginating. The spiral filament is broken into small fragments in the act of evagination. The fragments, which remain sticking to the inner membrane (now on the outside), are responsible for the broad, indefinite, spiral ornamentation on the outside of the evaginated tube. $\times 70$ diameters.

8 A portion of the evaginating ejaculatory apparatus at a slightly later interval than shown in figure 7. This shows the relation of the membranes in the evaginating position. $\times 70$ diameters.

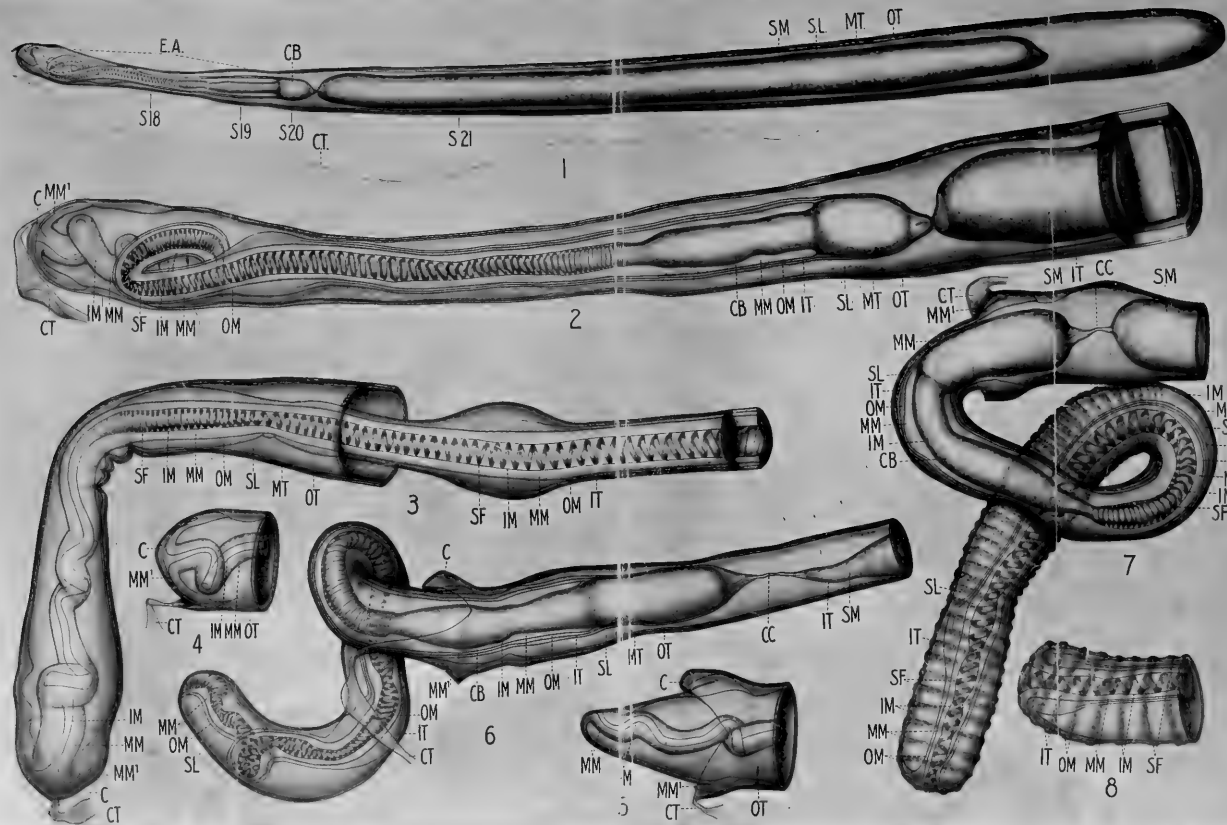


PLATE 2

EXPLANATION OF FIGURES

9 Extremity of the evaginating portion of a spermatophore at the instant the cement body has reached the end of the nearly evaginated ejaculatory apparatus. The inner membrane and spiral filament have broken from the tip of the cement body and are seen at the limit of ornamentation on the outside. The middle membrane adheres closely to the cement body, but the pressure from behind has not yet caused the cement body to change shape. $\times 70$ diameters.

10 A portion of the evaginated ejaculatory apparatus of the same specimen shown in figure 9, taken some distance from the oral extremity, at the point where the thickened portions of the outer membrane and inner tunic now lie. Evagination has turned these membranes back on the inside of the ejaculatory apparatus where they now form a reservoir wall into which the disorganized sperm mass is being forced. $\times 70$ diameters.

11 Extremity of the evaginating portion of the spermatophore an instant later than shown by figure 9. The pressure from behind has caused the adhering middle membrane to draw out the oral portion of the cement body at the sides. $\times 70$ diameters.

12 A whole spermatophore shown at a stage of ejaculation just a little more advanced than shown by figure 11. For convenience in placing on the plate the spermatophore is drawn as if cut in two parts. The position of the sperm mass which is being forced through the evaginated ejaculatory apparatus is shown. $\times 20$ diameters.

13 Extremity of the evaginating portion of the spermatophore shown in figure 12. This is an instant later than the stage shown by figure 11. The adhering middle membrane has drawn the cement body out to form a cap over the end of the sperm mass which is being forced against it. $\times 70$ diameters.

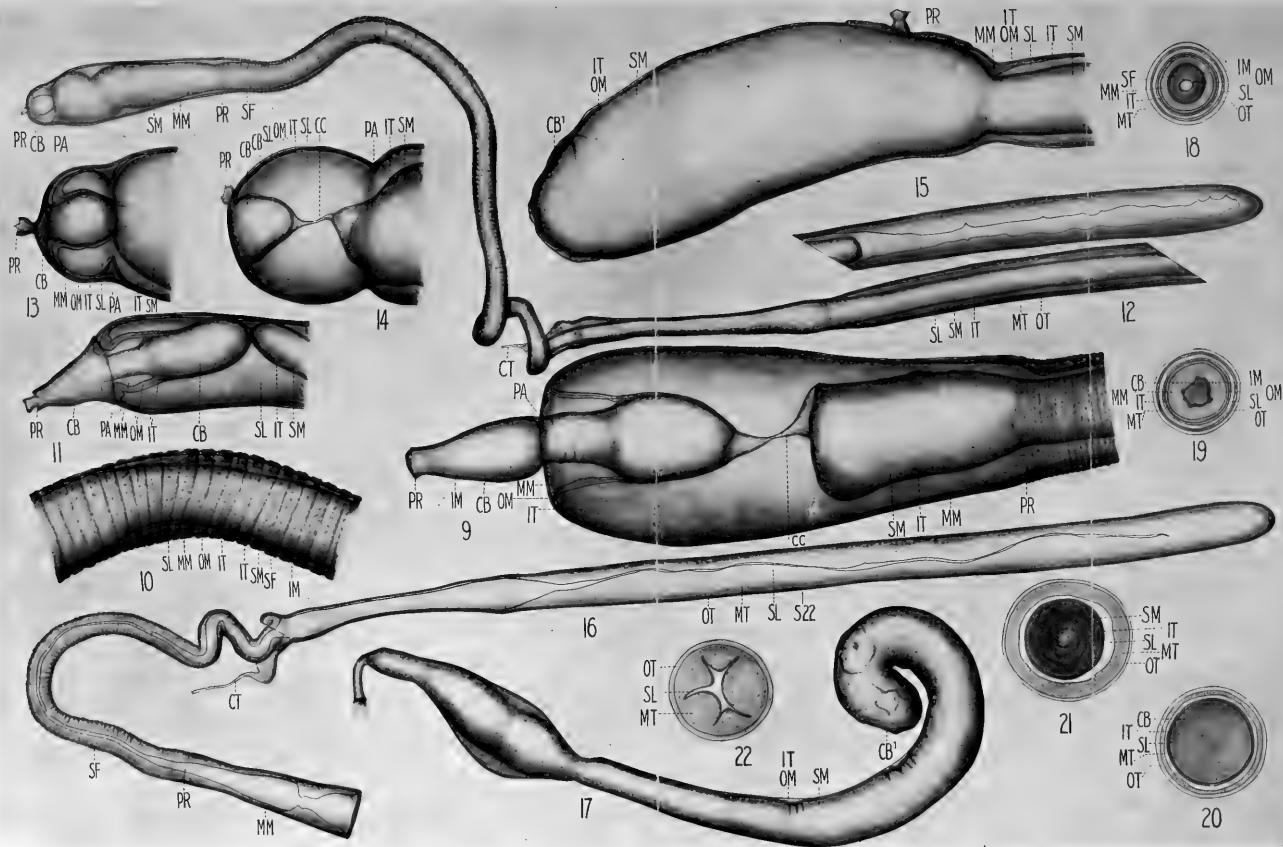
14 A slightly later stage than that shown by figure 13. $\times 70$ diameters.

15 Extremity of the evaginating portion of a spermatophore immediately after the pressure has caused the cement body, to which the middle membrane has adhered, to burst the inner membrane which has confined its viscid cement material. This act at once liberates the cement, which is spread over the end of the reservoir wall that encloses the sperm mass, and frees the reservoir wall, which consists of the outer membrane and inner tunic, now stretched and forced together so their individuality can no longer be distinguished, so it may slip out of the evaginated middle membrane, against which the outer membrane lies. $\times 70$ diameters.

16 The empty case, consisting of the outer and middle tunics, and the evaginated middle and inner membranes with the broken fragments of the spiral filament, after the sperm mass with the enclosing membranes and cement have been discharged. $\times 20$ diameters.

17 The sperm mass with the enclosing membranes and with the cement spread over the closed end, after being ejected from the case. This mass may be called the sperm reservoir. The walls consist of the stretched outer membrane and inner tunic, which are open at the pointed end. Here spermatozoa leaves as they are mixed with sea-water and become active. The cement hardens in sea-water and sticks the reservoir in place. The thickened portion near the opening, with the constricted portion immediately beyond it, is characteristic of the reservoirs. It may have something to do with the thickened portions of

(Continued on page 426)



the inner tunic and the outer membrane, but I am not certain this is the explanation. $\times 20$ diameters.

18 Transverse section of a spermatophore through the region of the spiral filament. For position see figure 1. $\times 70$ diameters.

19 Transverse section of a spermatophore through the region of the oral end of the cement body. For position see figure 1. $\times 70$ diameters.

20 Transverse section of a spermatophore through the region of the aboral end of the cement body. For position see figure 1. $\times 70$ diameters.

21 Transverse section of a spermatophore through the region of the sperm mass. For position see figure 1. $\times 70$ diameters.

22 Transverse section through the case of an ejaculated spermatophore. For position see figure 16. \times diameters.

PLATE 3

EXPLANATION OF FIGURES

23 and 23A Two continuous portions of the oral end of the same spermatophore dissected from a spermatophoric organ. The oral end of 23 was near the point C^1 , figure 32. The cement body was just beyond the point C^2 . The aboral end of the spermatophore (not represented in the figure) was near the distal end of the middle tunic gland. As the middle tunic is evidently almost completely formed, it would probably soon have been passed on to the outer tunic gland. $\times 70$ diameters.

24 Oral end of a forming spermatophore dissected from a spermatophoric organ. The oral end had passed through that portion of the outer tunic gland that connects with the middle tunic gland, and had passed into the finishing gland duct. The extreme end was near the point where this duct widens into the finishing gland. The aboral end of the specimen (not shown in the figure) was well back in the hardening gland and the cement body region was in that part of the outer tunic gland that extends up into the hardening gland. The outer tunic was elastic and would not adhere to a needle on that portion in the hardening gland, and was soft and sticky over the whole oral extremity. This portion had not been in the hardening gland and would not have entered it. $\times 70$ diameters.

25 Oral end of a forming spermatophore dissected from a spermatophoric organ. The oral extremity had reached the pointed end of the finishing gland and the aboral end (not shown in the figure) was in the finishing gland duct. The whole of the outer tunic, oral as well as aboral end, was elastic and would not adhere to a needle, but the spermatophore was not yet as turgid nor as small as those more fully formed. The shrinking of the oral end, the formation of the cap and the accompanying changes in the ejaculatory apparatus are the chief features to be understood. $\times 70$ diameters.

26 Oral end of a forming spermatophore dissected from a spermatophoric organ. It was contained entirely within the finishing gland and had its extreme oral end very near the opening of the spermatophoric duct. $\times 70$ diameters.

27 Oral end of a forming spermatophore dissected from a spermatophoric organ. The oral end had entered the spermatophoric duct. $\times 70$ diameters.

28 Oral end of a spermatophore dissected from a spermatophoric duct. A slight shrinkage, especially at the oral end, is the only change to take place in completing the spermatophore. $\times 70$ diameters.

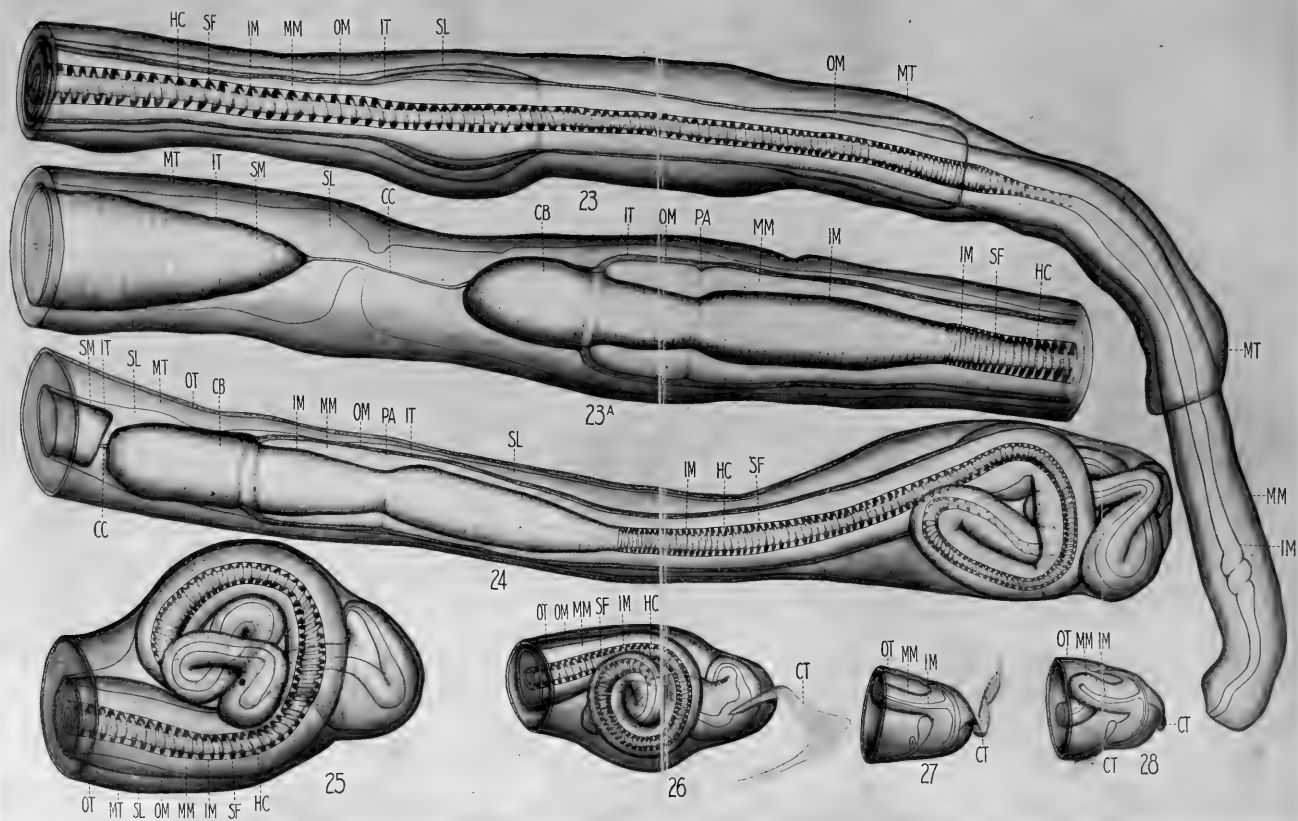


PLATE 4

EXPLANATION OF FIGURES

29 Spermatophoric organ seen from the surface that is free from the visceral mass. $\times 4$ diameters.

30 Spermatophoric organ seen from the surface that is applied to the visceral mass. $\times 4$ diameters.

31 Finishing gland end of a spermatophoric organ seen from the same position as that is figure 29, but showing cut ends of the parts exposed by cross-section. $\times 12$ diameters.

32 Semidiagrammatic view of a spermatophoric organ with the parts separated and the walls cut away to show the internal arrangements of the parts. The second division of the mucilaginous gland has had the wall cut away so as to expose the vas deferens as it approaches its entrance to this gland. The figure is made from the study of many dissections, and reconstructions from the study of sections of the organ.

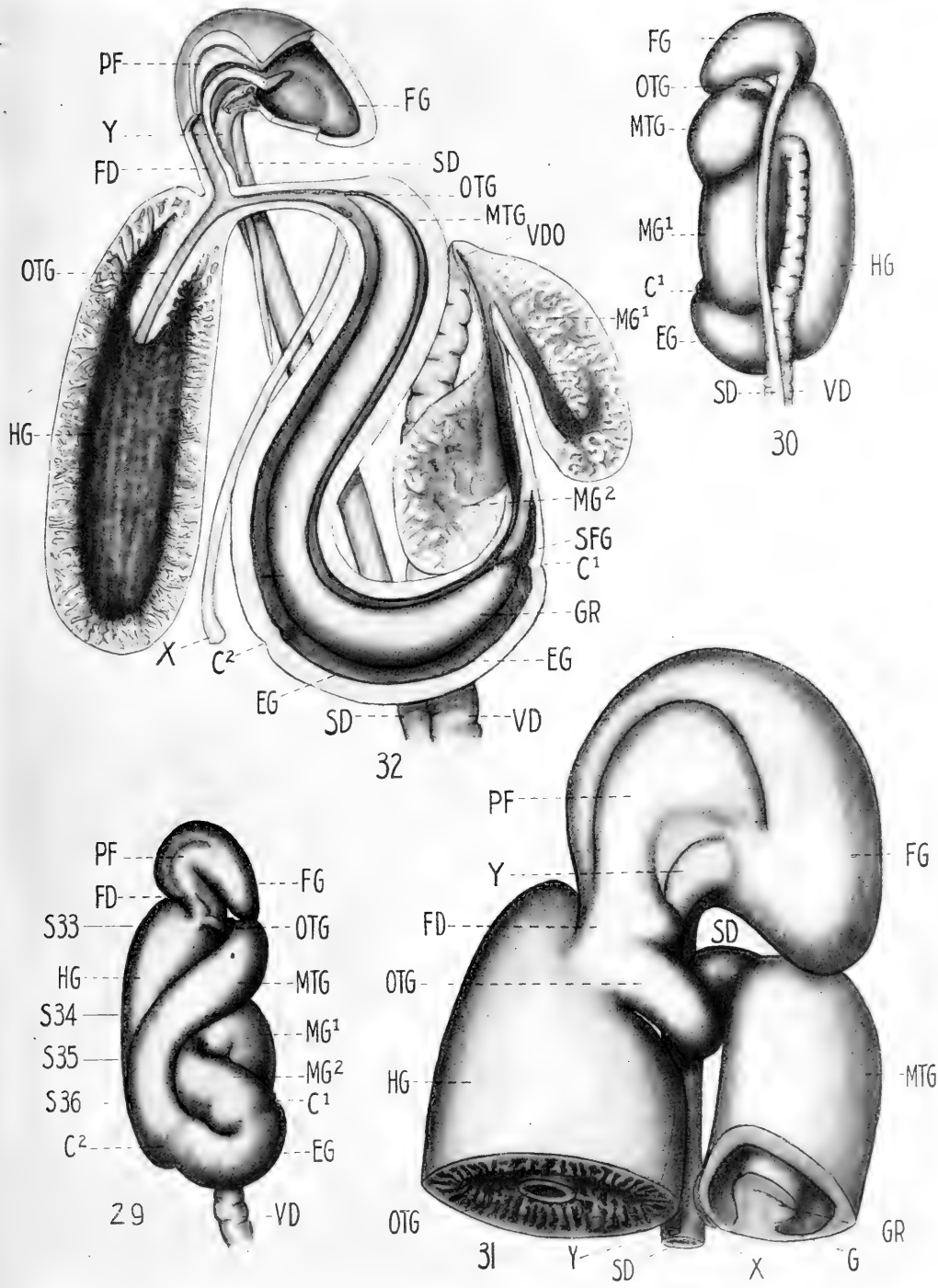


PLATE 5

EXPLANATION OF FIGURES

33, 34, 35, 36 Transverse sections of a spermatophoric organ. For positions of sections see figure 29. The spermatophoric sac, which is not a portion of this organ, is shown in the figures. $\times 12$ diameters.

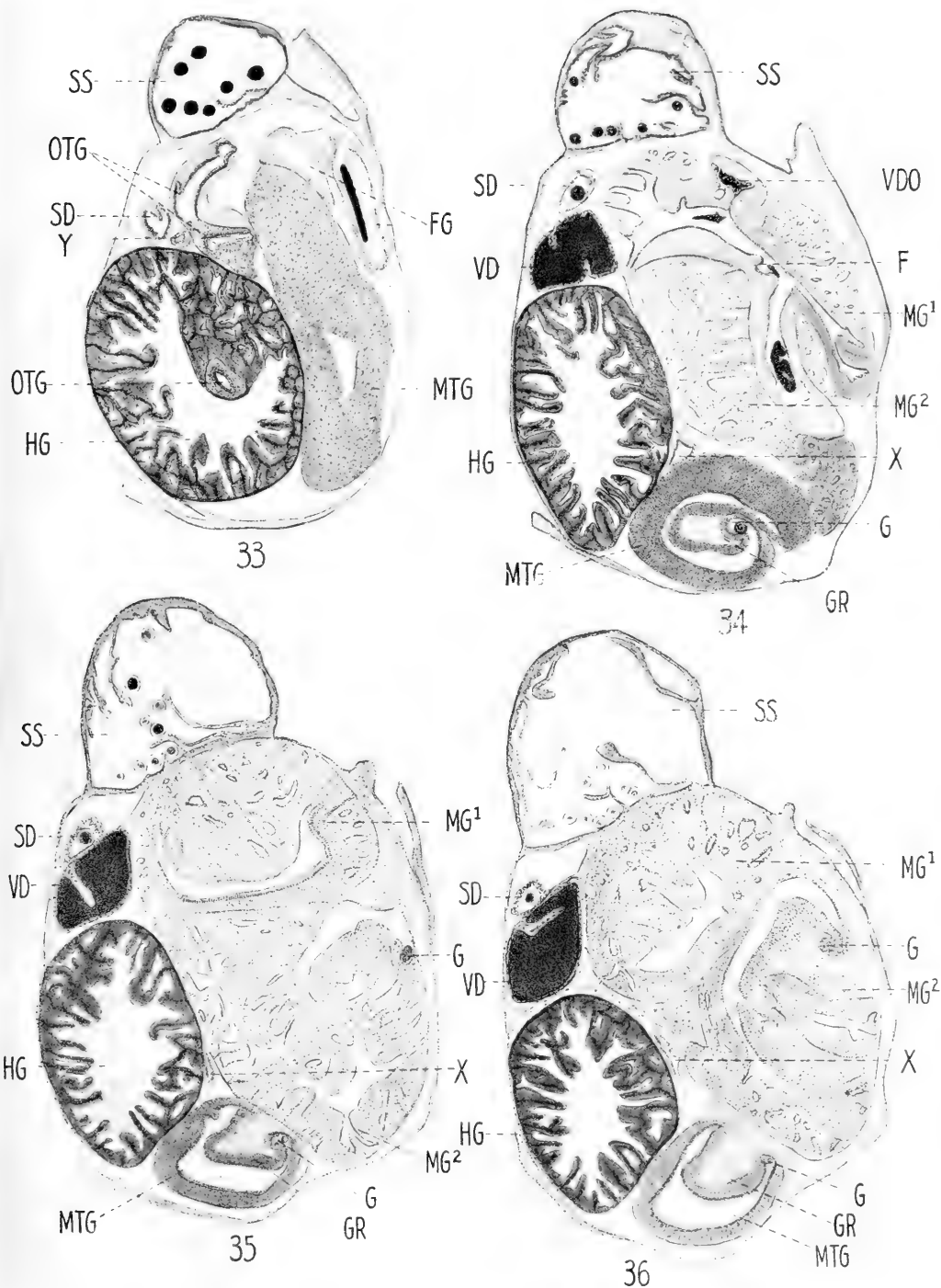


PLATE 6

EXPLANATION OF FIGURES

Diagrams showing the successive stages in the ejaculation of a spermatophore. For simplicity everything not necessary for understanding the process has been omitted from these figures. Thus the cap, the inner membrane and spiral filament, and the inner tunic are not shown.

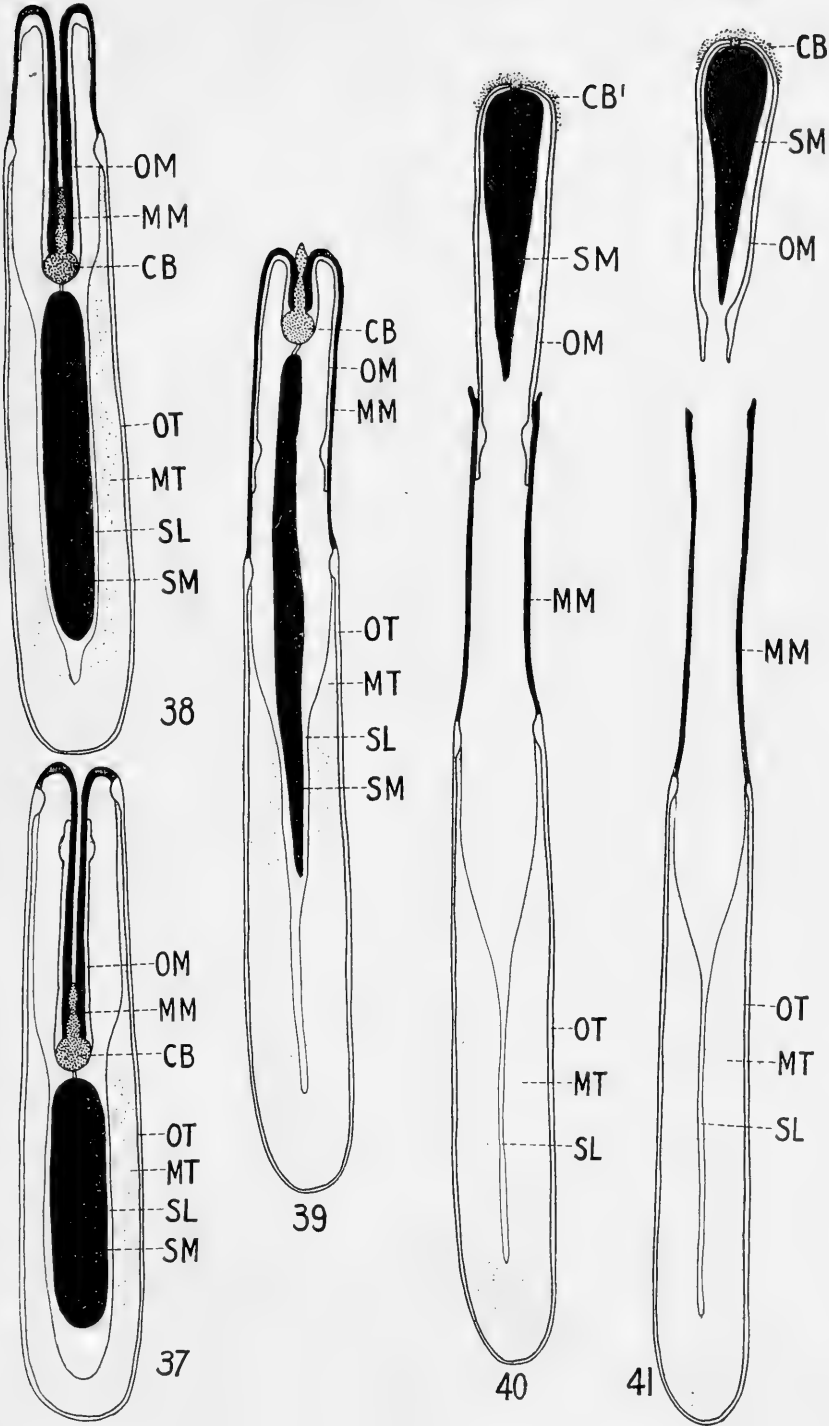
37 Diagram of the spermatophore ready for ejaculation.

38 Beginning of the process of ejaculation. The evagination of the ejaculatory apparatus, represented here by the middle and outer membranes.

39 Near the end of the evagination of the ejaculatory apparatus, showing the position of cement, sperm reservoir membrane (represented here by the outer membrane), and spermatozoa.

40 Sperm reservoir with filled spermatozoa, and the cement body ruptured.

41 The end of the process. The separation of the discarded case and the filled reservoir.



A COMPARATIVE STUDY OF THE CHROMOSOMES OF THE TIGER BEETLES (CICINDELIDAE)

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ONE HUNDRED TWENTY-SEVEN FIGURES (TEN PLATES)

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¹ Contribution No. 162. These investigations were carried on under the direction of Dr. Fernandus Payne to whom the writer is indebted for many valuable suggestions.

1. INTRODUCTION

The present paper follows the development of the germ cells of the male from the early spermatogonial stage to the mature spermatozoon, presents, so far as is possible, a study of the germ cells of the female, and considers the somatic divisions. The points of chief interest and importance are as follows:

1. The formation of syncytia in the early spermatogonia and their relation to the later stages of cellular development.
2. The marked difference between the early and last spermatogonial mitoses.
3. The numerical relations between the spermatogonial, oogonial, somatic, and first and second spermatocyte chromosomes.
4. The striking contrast between the behavior of the chromatin material in the growth period of the primary spermatocyte and that in the primary oocyte.
5. The presence and behavior of a double odd-chromosome.²
6. The great variety of abnormal mitotic figures and their relation to the centrosomes and spindle fibers.

2. REVIEW OF COLEOPTERAN CYTOLOGY

Although about seventy species of beetles are referred to in cytological literature as having been studied, the detailed history of the germ cells has been followed in only three or four species. Practically every worker seemed to consider his task completed when he had demonstrated the presence of an unequal pair of heterochromosomes.

Of the sixty-eight species listed by Harvey ('16) forty-five are attributed to Miss Stevens. Since she was primarily concerned with the sex-chromosomes, her observations on the other parts of the cell were only incidental and usually very meager. She gives the spermatogonial, oogonial, and first and second spermatocyte numbers of chromosomes in only seven species.

² Since this body behaves as the odd-chromosome in many forms, but in the present material is bipartite and may at certain times appear as two distinct chromosomes, the term 'double odd-chromosome' is used. Throughout this paper large 'X' is used to indicate the larger element of this bivalent body and small 'x' the smaller element.

The chromosomes of the beetles may be divided into three types, depending upon the behavior of the sex elements.

Type I

Stevens finds thirty species, ranging through thirteen families, which possess an unequal pair of sex-chromosomes ('heterochromosomes'). In each case the two members appear united on the first spermatocyte spindle, but separate and pass undivided to opposite poles. The union and separation of these members vary in different species. In *Haltica chalybea* (Chrysomelidae—Hydrophilidae according to Harvey, '16) the X and Y elements are widely separated in prophase and metaphase, but come together in early anaphase and then again separate and pass to opposite poles after the autosomes have divided. In *Doryphora decemlineata* and *D. clivicolis*, X and Y appear united in prophase and separate at various points on the spindle. Sometimes they seem not to separate at all, but the count in the second spermatocyte division shows that they do. In late anaphase the larger heterochromosome is often outside the polar mass, as is the odd-chromosome in the Orthoptera.

Wieman ('10) considers that Stevens has erroneously interpreted the behavior of the chromatin material in this form. He says:

The great similarity between the telophase of the first division, as represented by Stevens in figures 175 and 176 of her paper, and the corresponding stage in *L. signaticolis*, led me to examine the ovaries and testes of *D. decemlineata*. I found the nucleolus of the primary spermatocytes to accord with Stevens' description as far as the resting stage is concerned, *but that its unequal components separate in the first division, does not seem to be the case, and in this regard I cannot agree with her observations.*

In *Trirhabda virgata* the X is larger than the autosomes, while the Y is very small. In this case they are closely united to a plasmosome. At metaphase they separate and pass to opposite poles. In *T. canadense*, however, the two elements are more loosely attached to a plasmosome. In this condition they pass about half-way to one pole, then take their position on the spindle, and separate before the autosomes divide.

Nowlin's work ('06) on *Coptocycla aurichalcea* and *C. guttata*, though deficient in details, conforms with that of Stevens on thirty species, of which those considered above are types. In these forms Nowlin finds the unequal pair of heterochromosomes united, as usual, at metaphase; then they separate, pass to opposite poles, and divide normally in the second spermatocyte division.

Miss Nichols ('10) finds that in *Euchroma gigantea*, "the small heterochromosome is separated from the larger in the first division." Since she presents no further evidence and her figure 21 shows the two elements still united, her conclusion can have but little weight. The further meagerness of her observations is evinced by the fact that neither the spermatogonial nor the second maturation divisions are figured. Even the number of chromosomes is not given.

In *Cicindela primeriana* ('06) and *C. vulgaris* ('09), according to Stevens, the heterochromosome group is trilobed in the metaphase of the first maturation division. From this mass a small spherical chromosome separates and leaves a larger V-shaped one. Since each of these elements passes to opposite poles and divides normally in the second maturation division, the Cicindelidae are placed with this type. The observations set forth in the present paper, however, do not substantiate these conclusions.

It is observed that in each of the families Buprestidae, Cerambycidae, Chrysomelidae, Cicindelidae (according to Stevens, '06, '09), Coccinellidae, Melandryidae, Meloidae, Scarabaeidae, and Staphylinidae an unequal pair of heterochromosomes occurs. The larger member of the pair is the maternal homologue of the odd-chromosomes in the Dytiscidae, Elateridae, and Lampyridae.

The following is a typical fertilization formula for these families:

Chelymiorpha argus (Stevens, '06)

Sperm	Egg	
$(10 + Y)$	$+$	$(10 + X) = 20 + X + Y = 22 \text{ ♂}$
$(10 + X)$	$+$	$(10 + X) = 20 + 2 X = 22 \text{ ♀}$

Type II

As was noted above, *Doryphora decemlineata* (Wieman, '10) contains, in the growth period, a conspicuous basic-staining bipartite body. This element takes its position on the spindle as in the thirty or more species reported by Stevens. However, instead of the two parts separating, the entire body passes to one pole and divides normally in the last maturation division, giving dimorphic spermatozoa with sixteen and eighteen chromosomes. *D. decemlineata* is the only species of Coleoptera that has been recorded, prior to the appearance of the present paper, in which a bivalent sex-chromosome passes undivided to one pole. In this instance, however, neither the spermatogonial nor oogonial number is given.

As will be shown in this paper, the double odd-chromosome in the Cicindelidae takes the characteristic position on the first maturation spindle and then, in contradiction to the records of Stevens, passes to one pole undivided.

In the Carabidae, Hydrophilidae, Silphidae, and Tenebrionidae some species have an odd-chromosome; others, an unequal pair of heterochromosomes. The Cicindelidae and at least one Hydrophilid (Wieman, '10) possess a double odd-chromosome which passes to one pole of the first maturation spindle.

The typical fertilization formula of the last-mentioned forms in which the bivalent body passes undivided to one pole is as follows:

	Cicindelidae	(present paper)
Sperm	Egg	
	$10 + 10 + X + x$	$= 20 + X + x = 22 \text{ } \sigma$
$10 + X + x + (10 + X + x)$		$= 20 + 2X + 2x = 24 \text{ } \varphi$

Type III

The third type is characterized by the appearance of a single odd-chromosome. This type is represented by twelve species from seven families. Ten of these species were reported by Stevens. In all cases except two, the odd-chromosome passes undivided to one pole in the first maturation division and divides

The review of Harvey ('16) shows that no sex-chromosome is figured for a large number of species of beetles. In practically every instance, however, the observations were either meager or made by early workers. It might be concluded, therefore, that up to the present time the absence of the sex-chromosome has never been conclusively demonstrated in any species of beetles.

3. MATERIAL AND METHODS

The specimens used as a basis of this study were collected at various points in Indiana, and a record of their distribution and life habits is published in a separate paper (Goldsmith, '16 b). The most important points, however, in connection with the breeding habits of the tiger beetles should be mentioned, as a knowledge of life histories is of fundamental importance in making collections for cytological investigation. Shelford ('08) separates the life-histories of the members of this family into three types as follows:

(a) Eggs laid in the late spring or early summer; larvae hibernate usually in the third stage, pupate in the second summer; imagos emerge about a month after pupation, hibernate, and become sexually mature late in the third spring,—larval life lasts twelve to thirteen months, adult life ten months,—two years between generations.

(b) Eggs laid in mid-summer; larvae hibernate usually in the third stage, pupate in the following June; imagos emerge in early July and become sexually mature very soon,—larval life ten months, adult life two months, one year between generations.

(c) Eggs laid in mid-summer; larvae hibernate in the second stage, reach the third stage early in the second summer, hibernate again and pupate in the following May; imagos emerge in the early part of the third summer and become sexually mature soon,—larval life twenty-one months,—adult life two months,—two years between generations.

It will be noted that, from the standpoint of cytological work, it is practically impossible to discriminate between the form of type 'b' and type 'c,' as the adult life is the same, even though the larval life differs by eleven months. For convenience of discussion, therefore, I have elsewhere (Goldsmith, '16 b) used the term 'double-brooded' to apply to all forms under type 'b' and 'c,' having an adult life of two months. Since the imagos of

Cicindela repanda, *C. purpurea*, *C. ancocisconensis*, and *C. vulgaris* emerge in late summer or early fall, hibernate, and reappear in the spring, they are unquestionably classed under type 'a' and thus double-brooded. Specimens dissected from fall collections of these species proved very immature, but were very favorable for a study of the early spermatogonia and oogonia. On the other hand, the spring collections from these double-brooded forms were found to be of great value for a study of the later stages of the germ cells.

Since *Cicindela punctulata* and *C. sexguttata* appear in Indiana in late spring or early summer and die about eight weeks later, they are spoken of as single-brooded. Shelford's observations on the larvae and pupae of the latter form indicate that this species is in reality double-brooded. However, the young adults of the northern range do not dig their way out in the fall, but remain in the pupal burrows until spring. Since the single-brooded forms mature at once after appearance, there is a range of only about three or four weeks in each year in which favorable cytological material can be collected.

The technical side of the study of Coleopteran cytology is very difficult and disappointing in its results. All fixations in common use were tried under various conditions, but none proved entirely satisfactory. Several hundred specimens of Cicindelidae were dissected, resulting in but a few good fixations from each species. All of the best preparations were fixed in Flemming's fluid (strong). This method gave best results when the warm solution was dropped into the body cavity of the live specimen before dissecting out the gonads. After the fluid had had time to penetrate slightly all parts of the body, the gonads were removed and placed in cold Flemming for two hours. Even under these conditions good fixations were exceptional, and no explanation could be given for a good preparation when obtained. One of the most perfect fixations (*C. sexguttata*) was one of seventeen specimens collected, dissected, and treated under the same conditions. The other sixteen were absolutely worthless. Iron-haematoxylin, with orange G as a counterstain, when needed, was used most extensively.

4. THE SPERMATOGONIA

Of the five species presented in this study, only a few minor differences in the cellular behavior were found. The differences were not sufficiently great to warrant a separate discussion for each species. Unless otherwise specified, the descriptions and drawing are based upon *C. sexguttata*.

A. Syncytia

The anterior end of each bipartite testis is a loosely arranged tubular coil containing early spermatogonial cells. The youngest of these are crowded with cells having no perceptible cell wall (figs. 5, 6, and 7); the wide internuclear protoplasmic spaces are homogeneous save for a few scattered unknown chromatin staining bodies (figs. 5, 6, and 7). As the cells further mature, light streaks may occur here and there in the cytoplasm, appearing as cytoplasmic fibrillar bridges. With the increase in age and size of the cells, these bridges become more dense and assume a definite arrangement about a number of cells. This continues until the entire tubule is subdivided into a large number of syncytia—cysts containing cells without perceptible cell walls.

Wieman ('10) presents a study of the cyst formation in one of the Chrysomelid beetles, *Leptinotarsa signaticollis*, in which he concludes that the process is carried on by amitotic cells multiplication. He says: "At any rate, in the earliest stage at which the cysts can be recognized, they are filled with cells undergoing amitosis." Disregarding the controversy over amitotic divisions in the primordial germ cells, the fact that such divisions were found in the cyst when it is first recognizable, does not seem to justify the conclusion that these cells are fundamentally concerned in cyst formation. No such cell divisions were found in the formation of the testicular syncytia of the tiger beetles. In the stage represented in figures 5, 6, and 7 the syncytia are somewhat elongated, and contain from five to eight giant nuclei in cross-section or a total of from twenty to thirty nuclei.

As to the relation between the early spermatogonia and the syncytial membrane, we can only surmise. If, however, such a membrane does not exist prior to its apparent formation—and such seems to be the case—it does not seem justifiable to conclude that the containing cells of each cyst were all derived from a single primordial germ cell. Here again, Wieman seems to conclude prematurely that, “the contents of each cyst are the descendants of a single mother-cell.” Since the cysts were filled with cells when first recognized, it does not seem possible to determine whether or not all cells of a cyst were direct descendants of a single cell. In fact, if the membrane of the syncytium is indiscriminately formed among the early spermatogonial cells, as seems to be the case in the Cicindelidae, this conclusion cannot be justified.

These syncytial membranes become more and more defined and persist throughout maturation. At the close of the spermatogonial divisions, these membranes usually are separated at places by wide protoplasmic non-cellular spaces which increase in size throughout spermatogenesis.

The testis of the imago contains spermatogonial cells which are differentiated by neither a perceptible cell wall nor a syncytial membrane. Any region of such a testis may show various stages of cell division. Sometimes one or more neighboring cells in a prophase field may be in metaphase. This suggests that there is little relation between the adjacent cells with reference to their sequence of development. On the other hand, after the syncytial membranes are formed, it is true, as shown in figures 5, 6, and 7, that the stages of cellular development in each early syncytium are the same. This unity of cellular development persists until late in the maturation period. Furthermore, a cross-section of a testicular tubule usually shows as many distinct stages as there are syncytia represented. These results would suggest that the contents of each syncytium, rather than the cell itself, constitute a unit of cellular activity.

B. Early spermatogonia

In the early spermatogonial nuclei there is a fine chromatin network, not evenly distributed but more or less in clumps. In practically every instance it was either attached to, or most abundant near the nuclear wall and had a fibrillar connection throughout the nucleus. As the nucleus grows, this network becomes broken here and there, leaving the central area almost free from chromatin (figs. 1 and 5). Simultaneously and associated with the breaking of the central network, the chromatin aggregations become more conspicuous and come to lie nearer to, or in contact with, the nuclear wall. In some instances, however, one or more clumps of chromatin remain some distance from the nuclear wall, but in all observed cases, they were connected with this membrane by anastomosing fibrillar bridges (fig. 1). Since the small particles of chromatin are also deposited on the nuclear wall, this membrane soon becomes much more conspicuous than in earlier stages. The staining capacity continues to increase until the nucleus reaches its maximum size (figs. 1 and 2). This condition, as well as the formation of the spermatogonial chromosomes, is highly suggestive of the concluding stages of the growth period in the Hemiptera and other forms. It is interesting to note that the prophase spermatogonial nuclei are much larger than those in the corresponding stage of the first spermatocyte division (figs. 29 and 30). The mean diameter in case of the former is 11μ , while the latter measured only about 6μ .

The above chromatin aggregations are further differentiated by the smaller particles uniting with them, leaving the intervening spaces clear. This method of intensifying the nuclear wall by the addition of chromatin granules and the disappearance of it with the withdrawal of the chromatin material again suggest that "this membrane may be, at least in part, chromatic" (Goldsmith, '16 a).

The irregular chromatin masses now begin to suggest the shape of spermatogonial chromosomes (figs. 2, 3, and 4). These more or less definite but granular bodies assemble about the

central part of the former nuclear area and the granular cytoplasm crowds in from all sides. The spindle fibers now appear and the twenty-two compact chromosomes are drawn into metaphase (figs. 8, 9, and 10).

The shape and size of these chromosomes vary from the large asymmetrically armed V's to very small spheres. Intermediate between these two extremes are the hooked or J-shaped, the uniformly rod-shaped, the pointed rods or club-shaped, and the circular V's or U's of various shapes and sizes.

Although definite pairs of chromosomes can readily be recognized in every clear spermatogonial metaphase plate, the arranging of all the chromosomes into a paired series is very unsatisfactory. This is due principally to the fact that they vary somewhat in shape in different plates. Since the pairing is an arbitrary matter, the discussion on this point is confined to the larger pairs which are recognized with greater certainty. Pair 'A' (figs. 8 and 9) is composed of large V-shaped chromosomes constricted at the base and increasing in size from that point outward. The arms seem to be about equal in size, but each possesses its characteristic shape (fig. 10); one is somewhat crooked, having the concave side inward, while the other is club-shaped. The arms normally stand about 10° or 20° apart, but the angle of divergence may vary from zero to 170° . The V's which are opened widely are usually found with the apex at the periphery of the plate and with the arms extending left and right (fig. 13). They are oftentimes constricted at the apexes to such an extent that they appear, under low power, as two pointed rod-shaped chromosomes with the sharp ends touching. This condition is more often found when the arms of the V's are pressed almost together. This accounts for the large number of plates that seemingly present twenty-three and twenty-four chromosomes.

Although the hook (fig. 9, *B*) and pointed rod-shaped (fig. 9, *C*) chromosomes vary, they are readily recognized as pairs. The former vary from straight rods to hooks, while the latter vary from pointed clubs to blunt rods. The characteristic shapes of the remaining chromosomes are not sufficiently prominent to warrant a comparative study.

A study of the anaphase stage clearly shows that the diploid, and not haploid, number passes to each pole. It will be noted in figure 15 b that, even though the chromosomes have coalesced, the diploid number still persists. These observations corroborate those of Metz on the corresponding stages in the Diptera, but oppose the view of Lomen ('14) and Taylor ('14).

The position of spindle fiber attachment varies with the shape of the chromosomes. In case of the unequal armed V's (pair A) and the hooks or J's (pair B), the fibers are attached at the apex of the angle formed by the arms. In the open U's the fibers also seem to arise from the median region. In the straight rod chromosomes the spindle attachment is terminal.

C. Late spermatogonia

The last spermatogonial cells and mitotic figures, as well as the entire cysts, are characteristically different from those of earlier stages. The cysts are much more pronounced, having large intervening non-cellular spaces; they are very large and contain many more cells than in the earlier stages. The number of cells was counted in fifty typical cross-sections, and the average for each was thirty-seven. Since each cyst continues through about eight 5μ sections, the total number of cells in each cyst would approximate 250, allowing for those which might appear in two sections. However, the cells here are only 7μ in diameter, as opposed to 11μ in the former stages.

The secondary spermatogonial mitotic figures are much smaller than the primary. This renders an analysis of the spindle content much more difficult, as the same number of chromosomes is crowded into a much smaller space. It was impossible to study the chromosomes except at metaphase, and even here the entire plate could not be analyzed. Figures 12 and 13 represent the most favorable plates. Neither of these, even though they are clearer than revealed by the microscope, shows twenty-two chromosomes. There is no doubt, however, that the full number could be counted, were it possible to obtain sufficiently differentiated material. Further, the chromosomes of the late divisions differ in shape from those of the earlier.

The rods are shorter and thicker, the U's are more bean- or kidney-shaped, and the conspicuous V's are more widely open. These changes are due, no doubt, to the increased pressure and to the crowded condition.

5. GROWTH PERIOD OF THE PRIMARY SPERMATOCYTE

The spermatogonial telophase chromosomes, though drawn out and confused, show in cross-section a certain degree of individuality (fig. 15 b). At this point they still appear somewhat compact, but readily change to a woolly appearance (fig. 16 b).

In the earliest growth period the chromatic material presents itself as faint, delicately coiled threads, having no perceptible limiting membrane (fig. 17). Only under the most favorable conditions are the sex-chromosomes discernible. During the formation of the leptotene stage these fibers increase in size and staining reaction, and thus present a very crowded nucleus (figs. 18 and 19). The entire mass of chromatic threads now gradually contracts and culminates in a typical synaptic knot (figs. 20 and 21). This is usually spherical and lies against the nuclear membrane which made its appearance in the late pre-synaptic stage. In some cases the synaptic knot is very irregular or flattened (fig. 20) and extends across the central part of the nucleus. Here, as in case of the majority of presynaptic leptotene nuclei, the chromatin nucleolus cannot be identified with certainty.

The postsynaptic pachytene is inaugurated by the gradual loosening of the chromatic fibers of the synaptic knot. The outer loops, which seem to persist throughout synizesis, first recede from the chromatic bundle, giving room for the loosening of the central fibers. This proceeds until the nucleus is again crowded with chromatic threads (figs. 25 and 26), much coarser and less numerous, however, than in the former leptotene nucleus. A number of instances were noted in which the chromatin fibers did not loosen from the synaptic knot. In these cases practically the entire nuclear content formed a very dense sphere, and then the entire cell degenerated (figs. 23 and 24). As the nucleus

reaches the prophase condition without any perceivable split in the chromatin threads, the diplotene stage is imperceptible and the diffuse condition entirely lacking. The heavy, densely staining chromatic rods of the prophase stages seem to be derived directly from the pachytene nucleus. The woolly chromatic strands of the early prophase are gradually transformed into more definite late prophase chromatic bars (figs. 26 to 31). The most marked differences, however, between the early and late prophase cells are the gradual increase in nuclear size and the development of the nucleolus from an almost non-perceptible body to its conspicuous and characteristic late prophase form (figs. 29 and 30).

6. FIRST SPERMATOCYTE DIVISION

A. The autosomes

Near the close of the growth period, the cytoplasm seems to pass to one end of the cell, leaving the nuclear wall and the cell membrane almost or quite in contact, giving the cell an elongated, triangular appearance (figs. 29 and 30). A single centrosome is sometimes seen in the central part of this cytoplasmic mass some distance from the nuclear wall. The large, woolly, chromatin rods often give indications of polarization in the vicinity of the appearing centrosome (fig. 29). The chromatic nucleolus now assumes its characteristic bivalent (but unequal) appearance of the first spermatocyte mitosis. Upon the appearance of the spindle fibers and the breaking down of the nuclear membrane, the cell reassumes its somewhat symmetrical form, and the irregular chromosomes make their appearance (fig. 31). As the eleven first spermatocyte chromosomes take their position on the metaphase spindle, they represent almost as many types as there are individuals, but the shape and size of each is fairly constant at each corresponding stage of division. Figure 37 shows in detail the characteristic shape of the average chromosomes at metaphase and the approximate point of spindle attachment. Spindles showing all of these elements in the same phase are exceedingly rare, as the shape is constantly changing with the

progress of the division of the chromosomes. For example, many cases were found in which certain chromosomes would bear a marked resemblance to others, but as the division progressed, the characteristic shape would be assumed. This condition caused the various spindles to present, seemingly, a great variety of chromosomes. A study of the metaphase plate yields little results, as the number and shape of the visible chromosomes depend entirely upon the point of cross-section and the stage of development. The drawn-out chromosomes of late anaphase soon break and form the irregular chromatic masses of the early telophase (figs. 44 and 45). In late telophase (fig. 46) the spindle usually condenses and gives rise to a faint midbody.

B. The double odd-chromosome

The double odd-chromosome cannot be recognized in prophase on account of the confused condition of the autosomes; in later phases, however, it is very conspicuous (figs. 36, 39, 41, 42, and 43) and is surrounded by a clear area, leaving it seemingly free from spindle attachments (fig. 42). Its disposition is, therefore, left to the law of chance, and thus the body may appear at any point on the spindle. In metaphase it usually appears eccentric and in advance of the other chromosomes. On account of its position, it may be separated from the other chromosomes either by cutting the spindle crosswise or sagittally. This, no doubt, accounts for the fact that many of these bodies are found seemingly free in the cytoplasm, while innumerable spindles are found which seem to lack them (figs. 32 and 38).

Under ordinary staining conditions, the double odd-chromosome appears as a spherical chromosome attempting an unequal division, but when more stain is extracted, the bivalent nature becomes more apparent. This is especially true in the anaphase stages, when the body appears as a large flattened and a small spherical chromosome stuck together by achromatic material. The larger part of the element frequently shows an invagination in the central region opposite the point of attachment of the small member (figs. 39 and 41), which gives it the appearance of a small single and a large double chromosome. It was, no

doubt, the extreme of this condition which attracted the attention of Stevens ('09) when she suggested that, "In the first spermatocyte spindle [of *C. vulgaris*] the conspicuous elements are the trilobed heterochromosome group and a four-lobed or cross-shaped macrochromosome" (Stevens, fig. 88, reproduced fig. 40). The "four-lobed or cross-shaped macrochromosome" (fig. 40, *h*) is evidently an early stage in the formation of the ring chromosome (fig. 37, *h*, *i*, and *j*).

There is a remarkable similarity between the first spermatocyte chromosomes of *C. sexguttata* and those of *Coptocycla aurichalcea* (Nowlin, '06) and a number of forms worked by Stevens ('06 and '09). In the latter, however, the small and large elements separate in anaphase and go to opposite poles, thus giving two types of spermatozoa. One contains the large, and the other, the small element. In the case under consideration no evidence of separation has been found; while on the other hand it is very difficult, from direct observations, to establish the fact that such does not occur. However, the following facts seem to be sufficient to prove conclusively that this double element passes undivided to one pole. First, the number of second spermatocyte chromosomes is clearly ten and twelve (figs. 48 to 51). If the two parts of the double odd-chromosome should pass to opposite poles, all second spermatocyte divisions would be eleven, since the spermatogonial number is twenty-two. Second, two chromatin nucleolei are observed in the maturation stages of the female (figs. 95 to 99); while only one is found in the male. Third, there is no uniformity in the orientation of the double odd-chromosome (figs. 36, 39, 41, and 42). This body is often surrounded by a clear space, and this is seemingly free from fiber attachments (fig. 41). Fourth, the double odd-chromosome has been observed at or near the pole while the other chromosomes were in anaphase (fig. 43).

Brief reference should be made to some of the forms, exclusive of the beetles, whose sex-chromosomes behave somewhat similar to those of the Cicindelidae.

Wallace ('05) claims that in *Agalena naevia* two large elements pass undivided to the same pole in both the first and sec-

ond divisions, thus entering only one-fourth of the spermatozoa. The number of chromosomes are given as follows: spermatogonial 40, first spermatocyte 19 and $19 + 2X$, second spermatocyte 19 and $19 + 2X$. Boring ('07) doubts this observation.

Davis ('08) finds in *Arphia tenebrosa* two bodies which may pass to the same or opposite poles in the first division. In this case the number of chromosomes is given as follows: spermatogonial 24; first spermatocyte 13, and second spermatocyte 11, 12, and 13. If the observations of Davis be correct, this one animal is the only exception of this nature found among the Orthoptera.

In the pig (Woodsdalek, '13) the double X-element of the first spermatocyte spindle is smaller than the autosomes. Though the parts are not united, they pass to the same pole, eccentric and in advance of the other chromosomes.

In *Syromastes marginatus* (Wilson, '09 a) the 'double accessory' passes as a single body to one pole in the second division, while in *Phylloxera caryaecaulis* and *P. fallax* (Morgan, '15) and also in *Dolomedes fontanus* (Painter, '14) these elements pass undivided to the pole in the first division.

7. SECOND SPERMATOCYTE CHROMOSOMES

The second maturation division follows immediately after the telophase of the first, with no reconstruction of the nucleus. The representative number of chromosomes of this division is ten and twelve (figs. 48 to 51), while a number of plates were found which showed eleven and thirteen chromosomes. The cause of these aberrant numbers can be explained on the basis of faulty technique or precocious splitting and of overlapping and fusion of chromosomes. Observations show that the two elements of the double odd-chromosome which pass to the pole in advance of the autosomes in the first division, separate and act as single chromosomes in the second division. In the cells containing the twelve, the X elements cannot be definitely distinguished from the other chromosomes.

The second spermatocyte chromosomes present no such irregularities as are found in the first division, but more nearly

resemble the small chromosomes of the spermatogonia. When viewed from the side, the metaphase chromosomes appear exceedingly uniform, but a polar view usually shows one V-shaped and a number of irregular chromosomes. Although the present material is not especially favorable for a comparison of the diploid and haploid chromosomes, a close study of figures 48 and 50 might suggest that the V's and U's of the latter are the diploid pairs A and B, respectively.

The metaphase chromosomes appear on the spindle as bivalents (fig. 52), the elements of which pass irregularly to opposite poles (figs. 52 to 55). It is thus difficult to find an anaphase plate showing all the chromosomes in one plane. Late anaphases and telophases (figs. 55 to 58) are quite uniform, however, in comparison with the drawn-out and massed condition found in the first maturation division (figs. 44 and 45).

8. THE METAMORPHOSIS OF THE SPERMATIDS

At the close of the second maturation division the telophase chromatin mass is transformed into a dark spermatid nucleus, containing large granular strands of chromatin (fig. 59). This heavy network soon shows light areas, indicating a loosening of the nuclear content (fig. 60). The chromatin strands then become more conspicuous near the nuclear membrane, leaving the central part almost clear (figs. 61 and 62). They continue to condense until chromatin aggregations are formed, which resemble prophase chromosomes of very small cells (fig. 62). Whether or not the number of aggregations of chromatin at this point represent the haploid number of chromosomes we can only surmise. A large chromatin nucleolus is usually very prominent throughout these stages.

In the early spermatid the nucleus takes a position at one side of the irregular cell (figs. 59 to 61). The cytoplasmic part of the cell elongates, leaving only a very thin film on one side of the nucleus (figs. 60 to 63). In this large mass of cytoplasm and near the nuclear wall of the early spermatid can be observed, under very favorable conditions, a small area which seems to be less granular or fibrillar than the remaining cytoplasm. This

area gradually enlarges with the increase in length of the cytoplasmic tail, until a very conspicuous sphere is formed (figs. 60 to 63). Although this body could not be followed in other stages, it is assumed, by comparison with other forms, to be mitochondrial. The increase in the radius of this mitochondrial sphere places it in closer proximity to the nuclear wall. A faint filament—the first rudiment of the future axial filament—is now present (figs. 62 and 63). At the point of attachment of the filament to the nuclear wall, one or more small irregular bodies which are later concealed in the middle piece can usually be observed. Figure 63 represents the culmination of this entire process, both in the formation of the chromosome-like bodies of the spermatid nucleus and also in the development of the extranuclear sphere. The chromatin masses again become less compact and are distributed quite evenly throughout the nucleus. The nucleus now becomes more pointed at the end opposite the place of attachment of the axial filament (figs. 63 to 66). The cytoplasmic-like sphere and the cytoplasm elongate and condense to form the tail.

The junction between the extranuclear body and the basal part of the nucleus now becomes very dense (fig. 66). The following processes are so exceptional that it seems impossible for this plate to remain as the middle piece of the mature spermatozoon. Especially well differentiated material revealed the fact that this body was not a uniform plate as it usually appeared, but that it was made up of chromatin-staining bodies of various sizes. These chromatin-staining bodies, to which the axial filament seems to be attached, later appear more compact, move to one side, and then toward the anterior end of the nucleus (fig. 67). This change in position causes the filament to shift to one side and finally to come in contact with the wall of the elongated mitochondrial body (figs. 66 to 69). As the chromatin-staining mass proceeds forward (figs. 67 to 70), it gives indications of a bivalent nature and soon presents two conspicuous bodies which take their place near the forward end of the spermatozoon (figs. 71 and 72). As a result of this migration, the middle piece seems to become drawn out into a long

granular thread which becomes continuous with the axial filament. It can only be hoped that an interpretation of these two bodies and the significance of the migratory movement will be revealed by future researches on this and similar forms.

A third body, which may be the acrosome, makes its appearance at this time (fig. 71) and later fuses with the other two.

9. OBSERVATIONS ON THE OOGONIA

A. Oogonial growth period and prophase

The resting oogonia present little similarity to the corresponding cells of the male. There is no trace of a syncytium or even a cyst wall, but every cell is surrounded by its own conspicuous membrane (figs. 80 to 82). In view of the suggestion that each spermatogonial syncytium acts as a unit in the process of cell growth, we should expect a greater irregularity in the development of adjacent oogonial cells. This, indeed, is the case, for even though certain regions of an ovarian follicle show in general the same stage of development, it seems to be a matter of rare chance for adjacent cells to proceed with their development with the precise unity found in the corresponding spermatogonial cells.

The oogonial nucleus is much smaller than the spermatogonial and usually contains two well-defined chromatin nucleolei. The remaining chromatic material is scattered more or less in a fibrillar form throughout the nucleus. Upon the approach of the prophase condition, this chromatic material collects in masses, usually at the periphery of the nucleus. These chromatic aggregations gradually condense into the prophase chromosomes. The nuclear wall is now practically invisible as in the male (fig. 82).

B. Oogonial chromosomes

The oogonial number of chromosomes was practically established when the behavior of the double odd-chromosome in the male was determined. In order to further substantiate the earlier observations, special effort was made not only to obtain

the female count, but to extend the study of the cells of this sex as far as possible. Over 200 slides were made before a satisfactory count of the oogonial chromosomes was obtained. Even after the difficulty of poor fixation was partially overcome, the overlapping and irregular arrangement of the crowded metaphase chromosomes rendered the count practically impossible. The chromosomes of a single plate were never found in the same plane. Figure 85 shows two large chromosomes lying across the central part of the plate, while other figures (83 to 86) show a number of chromosomes lying at various angles to the metaphase plane. This suggests that they have passed the typical metaphase condition and are approaching early anaphase. If this be true, and there is a stage in which the chromosomes are arranged in a single plane, a sufficiently large number of divisions have been studied to justify the statement that the metaphase condition is practically instantaneous. The smaller chromosomes often lie in such close contact with the end of the larger that an especially good differentiation is required to distinguish the bivalent nature. A number of instances were also noted in which a small chromosome is above, below, or in contact with a larger one (fig. 84). A number of plates were also found which showed more than the normal number of chromosomes. The explanation of such cases is obvious from figure 83, in which the V's are almost perpendicular to the plane of the plate, thus a number of the arms are cut, causing each V to appear as two spherical chromosomes. A very careful study, however, of a large number of plates fully establishes the female number as twenty-four.

10. GROWTH PERIOD OF THE PRIMARY OOCYTE

A. Formation of leptotene threads

The reconstructing nuclei at the close of the last oogonial division differ from those of the earlier divisions. The telophase chromosomes remain for some time as compact, irregular chromatin masses, with woolly or fibrillar connections (fig. 95). As the cell begins to grow and the nuclear membrane becomes

more conspicuous, definite granular threads radiate from these chromatin masses (figs. 95 and 96). As the number and prominence of these chromatin threads increase, there is a corresponding decrease in the chromatic masses (figs. 96 and 97). This observation indicates that each leptotene thread is derived directly from an oogonial chromatic mass. This seems especially conclusive since the estimated number of leptotene threads approximate the number of oogonial chromosomes. In the typical leptotene nuclei (figs. 97 and 98) the long threads are twisted and irregularly arranged throughout the nucleus. They are usually attached to the nuclear membrane at one or both ends by an accumulated mass of chromatin-staining material. A cross-section not only shows a number of cut ends, but threads at various foci, depending upon the loop of the threads and angle of the section. It will be noted that these threads are rendered much more conspicuous by the granular enlargements—the chromomeres.

B. Sex chromosomes

The telophase of the last oogonial division shows all the chromatin masses of about the same density. However, in very early growth period, only one or two condensed bodies remain, and these show little relation to the chromatin fibers. This becomes especially apparent a little later when all other chromatin bodies have been transformed into the leptotene threads. In the earlier stages these bodies are usually compact (figs. 95, 96, and 97), but in later growth period they appear irregular and woolly (fig. 105).

Although the evidence is not conclusive that these bodies are the sex-chromosomes, it seems reasonable to assume that such is the case. According to the observational evidence illustrated in the fertilization formula, the cells of the female should contain twice the amount of X-chromatin as those of the male. That is, the male possesses $X + x$, while in the female cell there should be found $2X + 2x$. In accordance with these conclusions, observations further indicate that the chromatin content of the chromatin nucleolus of the female will approximate twice

that found in the male. In view of the fact that only two of the four female elements are visible, we might assume that the two large X's have fused to form the large nucleolus, and the two small x's to form the small one. The great variation, however, in the size of these two bodies tends to weaken these assumptions.

C. Bouquet, synizesis, and later stages

It has been noted that in the typical condition the leptotene threads are scattered loosely throughout the nuclei and may be attached to the nuclear wall at any point. Immediately following this typical leptotene condition, the nuclear wall on the one side becomes free from leptotene threads (fig. 99). At this time more threads than usual are attached by only one end, the other end floating free in the cell sap. This free end soon finds its way to the 'polarized' side of the nucleus where the opposite end is usually attached. By this method the large loops of the bouquet stage are formed, and the nucleus is cleared on one side of chromatin fibers (fig. 100). This method also clearly accounts for the fact that loops, rather than the ends of the leptotene threads, extend outward from the chromatin mass in the bouquet stage. As the loops are never drawn tightly together, there never appears a compact bouquet as described in other forms. Figure 101 is perhaps the most typical case found. No indications of a pairing of these threads were observed.

From the bouquet stage the threads emerge in broken pieces of more or less faintly stained chromatin rods (figs. 102 to 104). These appear very irregular and feathery, until the stage represented in figure 105 is reached. In this and later stages the chromatin material is scattered uniformly throughout the large nucleus in the form of faint anastomosing aggregations.

11. THE SOMATIC CELLS AND MITOSES

The follicular tissue of the ovaries proved very satisfactory for a study of the somatic cells. Although the majority of the cells are in a resting condition, mitotic divisions are compara-

tively abundant. The active cells show little indication of rhythm of cell activity.

The somatic resting cells (fig. 74) are much smaller than the germ cells. They usually possess two irregular feathery nucleoli. The chromatin material is scattered somewhat uniformly throughout the nucleus with here and there slight feathery aggregations of fibrillar material. The nuclear wall is very conspicuous, while the cell wall is somewhat less apparent. The entire cell, in case of the ovarian follicles, is rectangular and flattened, caused by the normal growth of the ovary. The prophase stage is inaugurated in the usual way by the gradual accumulation of chromatin material at various points in the nucleus and by a further transformation of these aggregations into irregularly shaped chromosomes. The formation of the prophase somatic chromosomes differs from that of the germ cells, especially in the testis, in that the chromatin aggregations are formed indiscriminately throughout the nucleus rather than in contact with or near the nuclear membrane. The somatic cells are so small that a satisfactory study of the prophase chromosomes is impossible.

Although the metaphase chromosomes were very difficult to study on account of the crowded and flattened condition of the cells, a number of plates were found in which the theoretical number, twenty-four, could be definitely established (figs. 76). The somatic chromosomes possess, in general, the characteristics of those of the germ cells, but show much greater variations in size, shape, and general arrangement. This is largely due to the crowded condition of the growing ovarian tissue. Many instances were noted in which the lateral pressure had been sufficiently great to force the metaphase plates into an extremely elongated form. Regardless of the variation in size and shape, a number of pairs of somatic chromosomes can be definitely determined. This observation indicates that the chromosomes of the somatic and germ cells possess the same general characteristics and that the somatic number in the female is the same as the oogonial number (figs. 83 to 86). The anaphase condition is characterized by a very early fusion of the chromosomes

(figs. 78 to 79). A few anaphase cells were found in which the chromosomes stood apart (fig. 77), but the number (diploid) was too great to permit a detailed study.

The late telophase chromosomes pass directly into the diffuse condition characteristic of the normal resting cell. There seems to be no further changes until the prophase chromatin aggregations are formed. No indications of synizesis (as Taylor, '14, p. 391, finds in the somatic cells of *Culex pipiens*) were observed in well-fixed material.

12. ABNORMAL MITOSES

In a number of specimens a variety of abnormalities was noted in the first spermatocyte mitoses. Figure 119 shows a typical multiple chromosome group. The normal number of chromosomes for this division is eleven, but twenty-two are clearly shown in this plate. Abnormalities of this type have been reported a number of times from other material. Metz ('16) finds (in the Diptera, notably in *Sarcophaga* and *Funcellia*), "certain cases of multiple chromosome numbers (tetraploid, or higher multiple). In these cases corresponding chromosomes were associated in prophase in aggregates of four, eight, etc., instead of being arranged in pairs." Wilson ('06) reports in *Anasa tristis* a number of oogonial cells containing forty-four chromosomes, when the normal number is twenty-two. He suggests that the presence of these multiple chromosome groups is due to the fact that, "all the chromosomes divided once without the occurrence of cytoplasmic division." Wilson also finds nine chromosomes in *Lygaeus turcicus* and in *Coenus delius*, when eight is the normal number. He says, "the presence of this additional chromosome is probably due to a failure of synapsis between two of the spermatogonial chromosomes which normally conjugate to form a bivalent body, and it is evidently to be regarded as an abnormal condition."

Randolph ('08) finds in the earwig, *Anisolabis maritima*, occasional giant nuclei with double the normal number of chromosomes. There also occur in the first spermatocyte divisions

of this material, tripolar or multipolar spindles, which probably explain a certain irregularity in the number of chromosomes.

Figures 120 to 127 show the extreme varieties of multipolar spindles found in the material under consideration. Figure 120 shows a first spermatocyte telophase more than twice the normal size. Size relations seem to indicate that figure 124 is an anaphase of this same condition. A large number of spindles (figs. 121, 122, and 125) showed indications of being absorbed in the cytoplasm. This destruction seemed always to take place about the time the chromosomes were in anaphase, as no telophase multipolar spindles were found. Even though individual chromosomes, or rather chromatin bodies, were found near the pole, the central region still held other chromatin bodies, which seemed to be attracted equally by all poles (figs. 122, 125 to 127). In figure 127 is a collection of irregular bodies being acted upon by six different centrosomes.

Figure 117 shows an elongated abnormal spindle, the fibers of which are bent around another abnormal spindle (fig. 118) shown in cross-section. The spindle in figure 118 stands perpendicular to the plane of that in figures 116 and 117.

An attempt to ascribe a cause for these abnormal processes would be, in general, very unsatisfactory. Randolph ('08) says, "In one case of abnormal spindle it is known that the material came from an earwig which had very recently molted; and it is possible that there is a connection between the two facts." Since molting is a normal process, and further since only one abnormal spindle was observed in one specimen, it seems premature to suggest even a possible association of the two facts.

It has been suggested that the chromosomes of the primary spermatocyte divisions in the tiger beetles are crowded and very irregular. This crowded condition was characteristic of all species studied. In many cases the chromosomes were so interlaced that the whole spindle presented a very abnormal appearance. In fact, it may be possible that the large number of abnormal mitotic figures found in the first spermatocyte division were conditioned partly by the crowding and interlacing of these irregular chromosomes.

13. SUMMARY

1. The behavior of the chromosomes of the beetles has been divided into three types; each is represented by a typical fertilization formula.

2. The early spermatogonia possess neither perceptible cell walls nor syncytial membranes. However, the latter soon forms and divides the testicular tubule into definite syncytia. All the cells in each syncytium are in exactly the same stage of development. This synchronism is broken, however, in the late maturation divisions after the cell walls become apparent. These observations would suggest that the contents of each syncytium, rather than the cell itself, constitute a unit of cellular activity. The early oogonia possess very definite cell walls, there being no indication of a syncytium or a cyst.

3. The spermatogonial number of chromosomes for each of the five species studied is twenty-two. The oogonial and the female somatic number is twenty-four each. Two distinct types of spermatogonia were found. The late spermatogonial cells are much smaller and stain more intensely than do those of the earlier divisions. The chromosomes of the late divisions are crowded and very difficult to figure.

4. Definite pairs of chromosomes are readily recognized in every clear spermatogonial, oogonial, and somatic metaphase plate.

5. The eleven first spermatocyte chromosomes are very irregular in shape and especially difficult to figure. Autosomes in the form of complete and incomplete V's of various sizes, rings, hooks, and rods were figured from side views of the spindles. The secondary spermatocyte numbers of chromosomes are ten and twelve. They are much more uniform than those of the first division.

6. The 'sex-chromosome' appears on the first spermatocyte spindle as a double body, the two elements (X, x) of which are very unequal in size and loosely united.

These elements neither divide nor separate in the first division, but pass to one pole in advance of the autosomes, giving

secondary spermatocytes, with ten (10) and twelve ($10 + X + x$) chromosomes, respectively. In the second division the components of the bipartite body separate, and both divide in this division with ten and twelve chromosomes.

7. The germ cells of the female seemed to contain approximately twice the amount of X chromatin as those of the male. This is in accordance with the fertilization formula considered in the text. The behavior of the chromatin of the growth period was followed through the leptotene, bouquet, and synzesis stages, to the breaking up of the late synaptic threads to form the faintly staining chromatin masses, characteristic of the prophase egg nucleus.

8. A number of abnormal mitotic figures were observed of which the following are types: metaphase plates containing multiple chromosome numbers, abnormally large telophases, spindles being absorbed in the cytoplasm, dipolar spindles containing only one large chromatin elements, and spindles with three, four, and six poles.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

Spermatogonia

- 1 Early resting spermatogonial nucleus.
- 2, 3, and 4 Formation of prophase spermatogonial chromosomes.
- 5, 6, and 7 Syncytia containing resting nuclei (fig. 5), prophase nuclei (6), and mitotic figures (fig. 7).

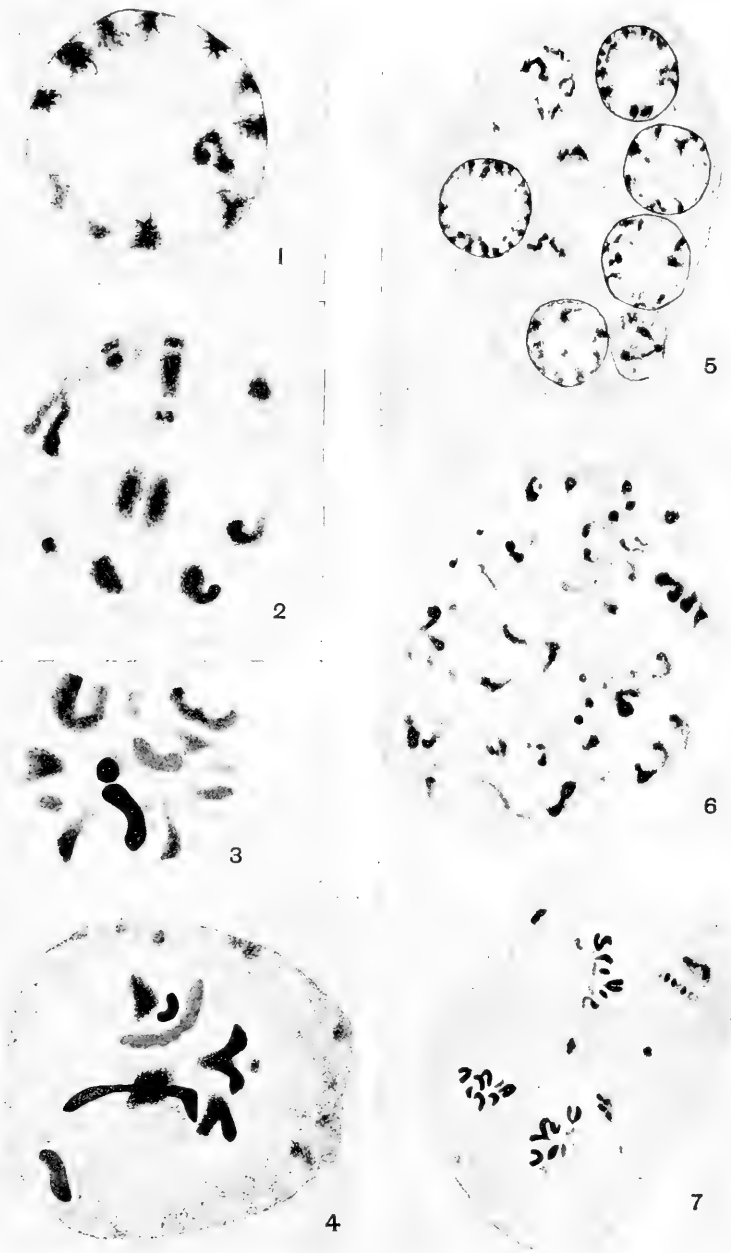


PLATE 2

EXPLANATION OF FIGURES

Spermatogonia—*Continued*

8, 9, and 10 Metaphase plates of the early spermatogonia. Figures 8 and 9 show the approximate pairing of the chromosomes.

11 Side view of the same stage.

12 and 13 Metaphase plates of the late spermatogonial mitoses.

14 and 15 Late anaphases showing the chromosomes approaching the poles

15 b Cross-section of an early telophase.

14, 15, and 16 Formation and development of midbodies.

16 a Late spermatogonial telophase showing the formation of the cell wall by the midbodies.

16 b Cross-sections of the nuclear region of the same stage showing the woolly appearance of the chromatic bodies.

Spermatogonial growth period

17 The diffuse postspermatogonial stage.

18 Early leptotene nucleus.

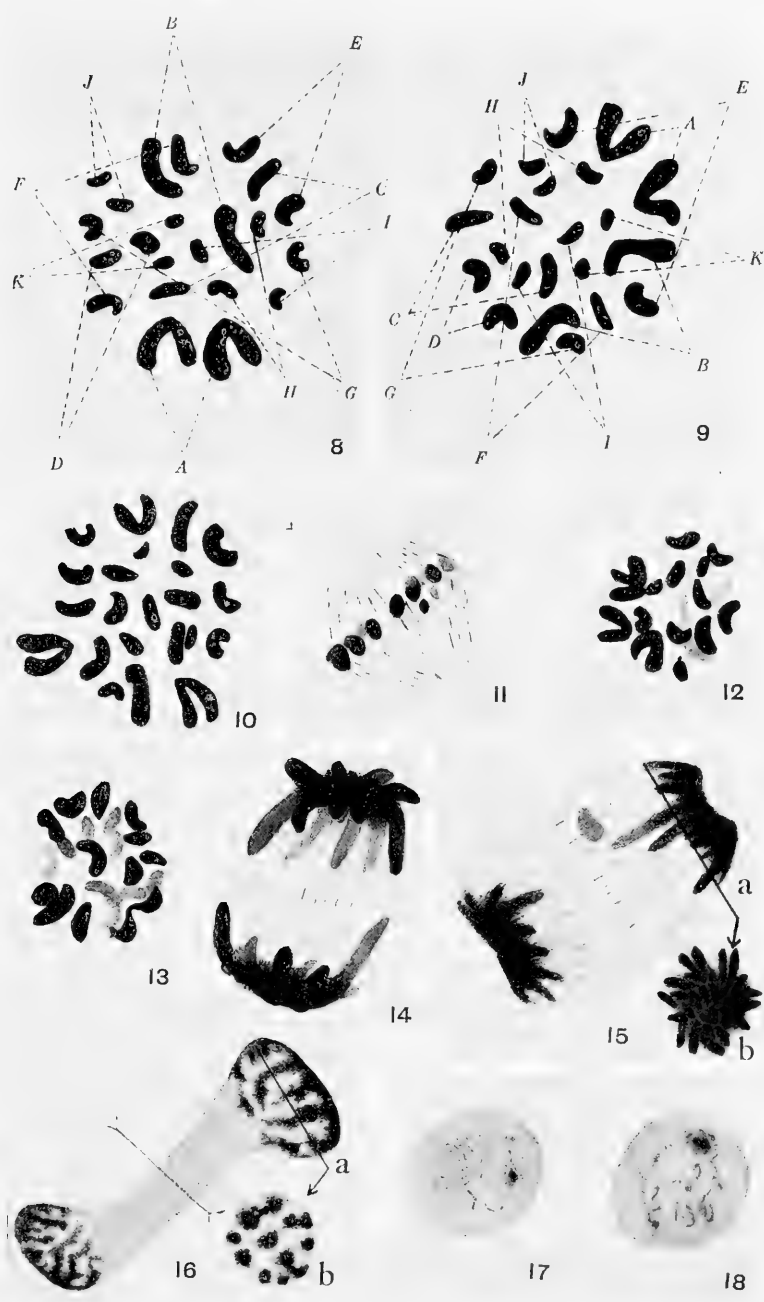


PLATE 3

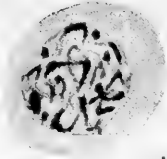
EXPLANATION OF FIGURES

Growth period—*Continued*

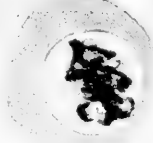
- 19 Later leptotene showing the process of unwinding and expansion completed.
- 20 and 21 Two cells in synizesis.
- 22 and 25 Pachytene nuclei.
- 23 and 24 Degenerating cells.
- 25 to 28. Early prophase nuclei. The sex-chromosomes usually appear in this stage as a more or less spherical body.
- 29 and 30 Later prophases showing elongation of the cell and position of the early centrosome. The nucleus has taken a peripheral position. The sex-chromosomes have become more conspicuous and very irregular in form.

Primary spermatocyte

- 31 Prophase chromosomes being drawn into metaphase position.
- 32 Side view of a first spermatocyte metaphase.
- 33 Polar view of the same.



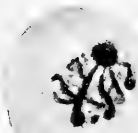
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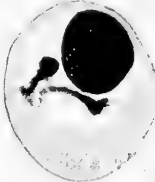
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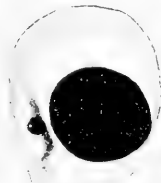
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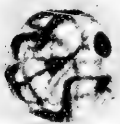
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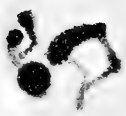
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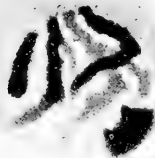
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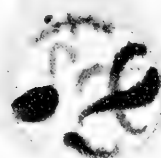
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PLATE 4

EXPLANATION OF FIGURES

Primary spermatocyte—*Continued*

34 and 35 Polar views of two first spermatocyte metaphase plates.

36, 38, 39, 41, 42, and 43 Early and late metaphase spindles showing the irregularity in shape of the autosomes and the position of the 'double odd-chromosome' (X).

37 Various forms of first spermatocyte chromosomes drawn from typical metaphase spindles. *b* is a later stage of *a*; and *d* a later stage of *c*; *h*, *i*, and *j* are stages in the development of the ring chromosome shown in figures 38 and 41.

40 First spermatocyte spindle of *C. vulgaris* (Stevens, '09, fig. 88), showing the "Trilobed heterochromosome group (x) and a four lobed or a cross-shaped macro-chromosome (h)." Compare X with X in figures 39, 41, and 42; also note the similarity between *h* in figures 40 and *h* in figure 37.

44 and 45 Typical late anaphase spindles.

46 Telophase.

Secondary spermatocyte

47 An exceptional secondary spermatocyte metaphase with eleven chromosomes.

48 A typical metaphase containing twelve chromosomes.

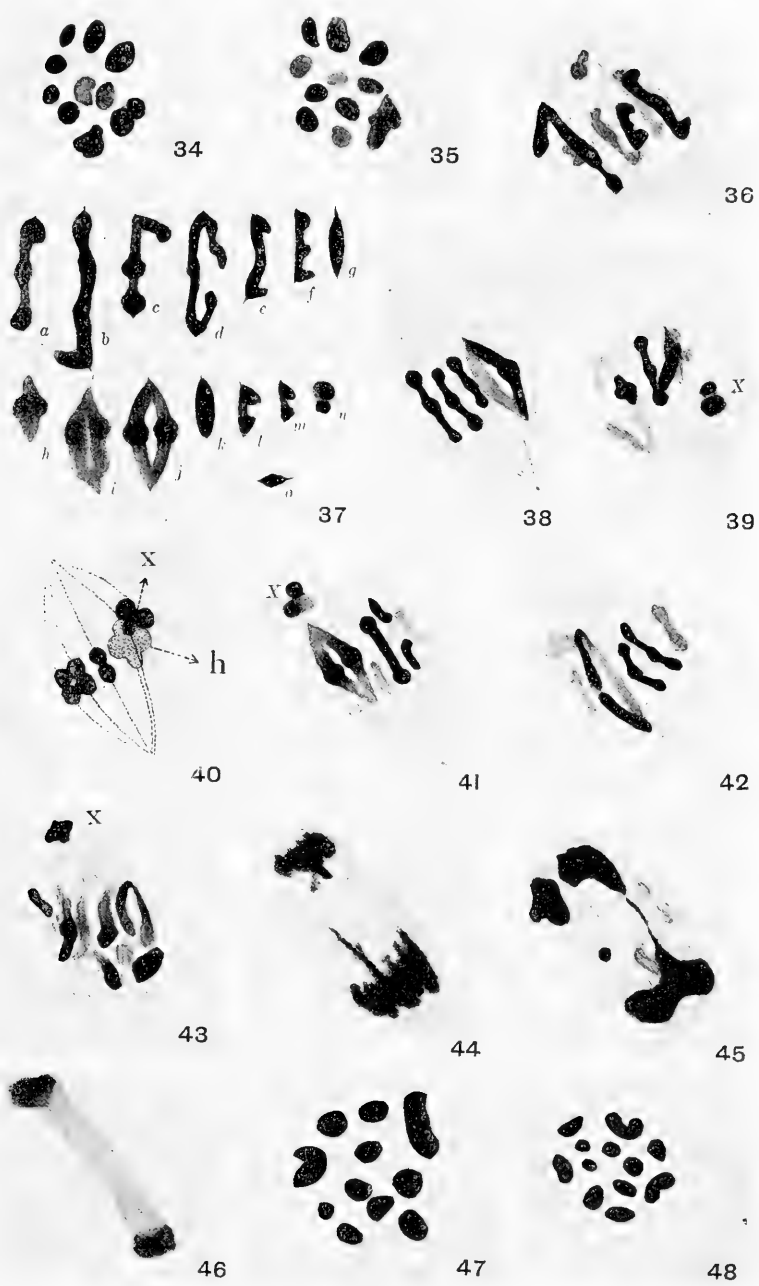


PLATE 5

EXPLANATION OF FIGURES

Secondary spermatocyte—*Continued*

- 49 Secondary spermatocyte metaphase—twelve chromosomes.
- 50 and 51 Secondary spermatocyte metaphases—ten chromosomes.
- 52 A typical secondary spermatocyte metaphase spindle, side view.
- 53 and 54 Early anaphases.
- 55 Later anaphase.
- 56 and 57 Telophases.
- 58 An exceptional telophase.
- 59 Late telophase showing the reconstruction of the two daughter-cells.

Metamorphosis of the spermatids

- 60 Early spermatid showing loosening of nuclear content and also first appearance of the supposed mitochondria in the cytoplasm.
- 60 to 63 Development of the 'mitochondrial mass' and elongation of the cytoplasm. Condensation of chromatin to form definite chromatin bodies characteristic of the spermatid nucleus.
- 63 A very typical stage at the conclusion of the formation of the mitochondrial mass. The nucleus at its maximum size showing indications of elongation.

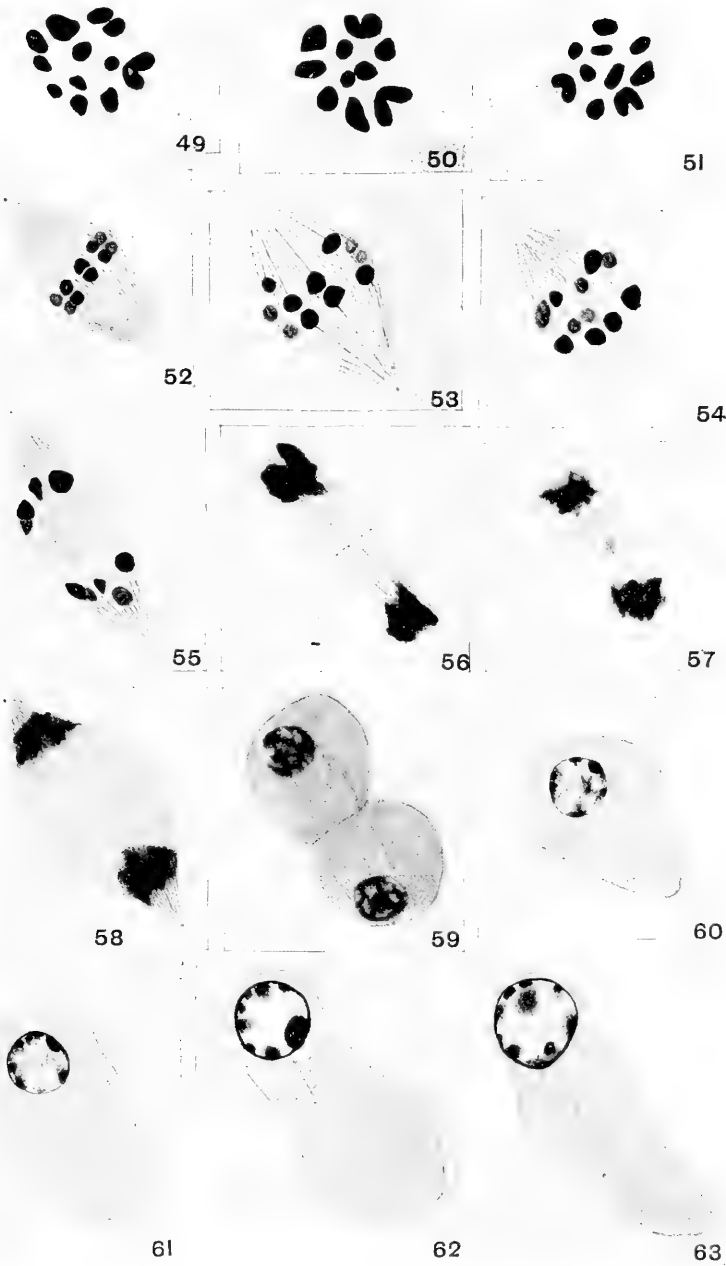


PLATE 6

EXPLANATION OF FIGURES

Metamorphosis of the spermatids—*Continued*

64 and 65 Elongation of the spherical nucleus and extranuclear body, to form the typical head and cytoplasmic-like body shown in figure 66.

66 to 72 Further transformation of the spermatid into the spermatozoan, showing method of elongation, disappearance of the neck plate and cytoplasmic body, and the diffusion of the chromatin. Migration of the two unknown chromatin-staining bodies to the anterior end of the spermatozoan, resulting in the production of a long, granular fiber (figs. 69 and 70) from the plate-like middle piece (fig. 66).

73 Mature spermatozoan showing the 'false' head. The true head constitutes, perhaps, the entire drawing, the tail being many times longer than the illustration.

Somatic mitosis

74 A resting somatic cell. All somatic figures are from ovarian follicles.

75 Side view of a metaphase spindle.

76 Metaphase plate—twenty-four chromosomes.

77 An exceptional anaphase.

78 and 79 Early telophases.



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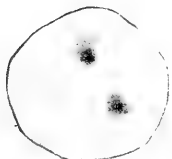
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PLATE 7

EXPLANATION OF FIGURES

Oogonia

80 Resting oogonial cell showing the two chromatin nucleoli and the arrangement of the chromatin material. A very conspicuous cell membrane is found in all the early oogonial cells.

81 Early prophase showing the newly formed chromatin aggregation.

82 Formation of prophase chromosomes—nuclear wall practically invisible.

83 to 86 Oogonial metasphase plate showing the overlapping and irregular arrangements of the chromosomes (figs. 83 and 85, *vulgaris*; 84 and 86, *ancocisconensis*).

87 Late metaphase, side view (*punctulata*).

88 Anaphase showing first visible appearance of the granular enlargements of the spindle fibers which form the 'Zwischenkörper.'

88 to 92 Successive stages in the development of the 'Zwischenkörper.'

90 to 92 Typical telophase oogonia, showing relation between the developing Zwischenkörper and the cell wall.

93 Daughter-nuclei reconstructed before the complete division of the cell or the disappearance of the Zwischenkörper.

94 Midbodies surrounding a bundle of spindle fibers lying free in the cytoplasm.

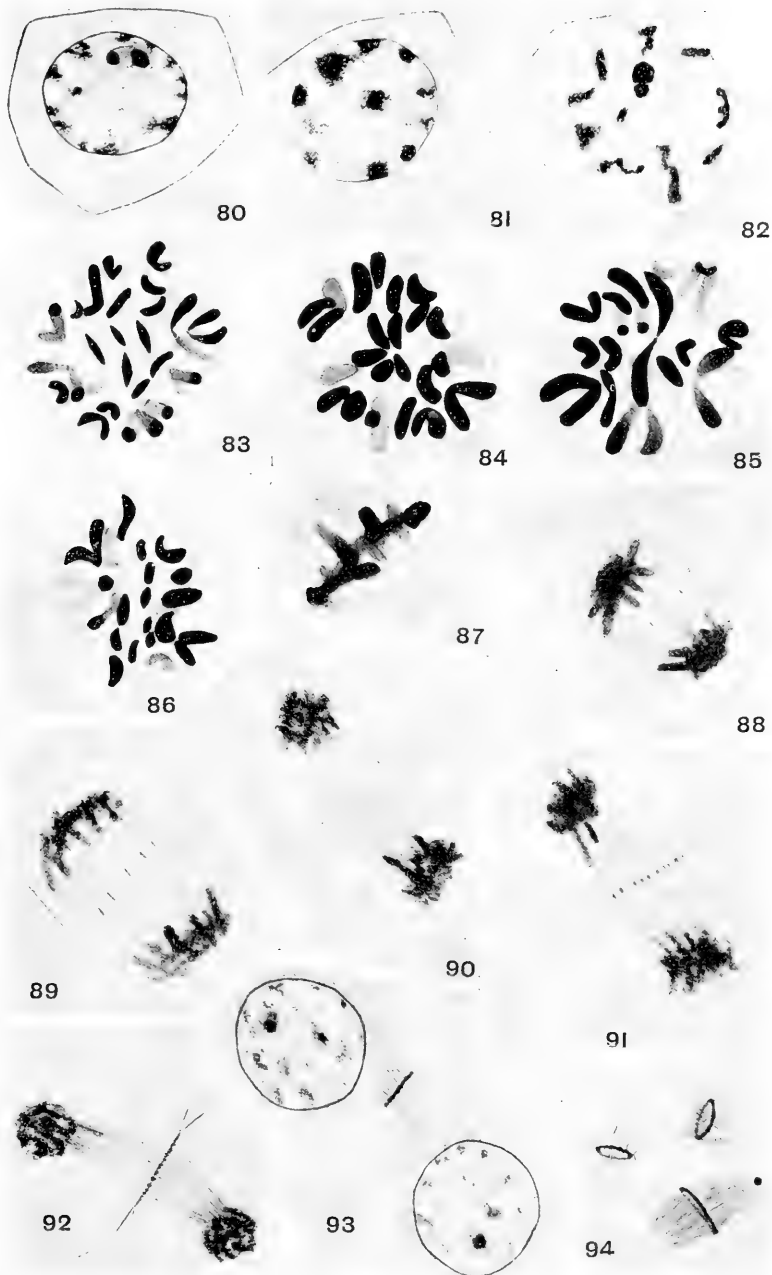


PLATE 8

EXPLANATION OF FIGURES

Oogonial growth period

95 Leptotene threads forming from the chromatin masses following the last spermatogonial division. Chromatin nucleoli plainly visible.

96 Further spinning out of the chromatin masses to form leptotene threads.

97 Typical nuclei of leptotene stage.

98 and 99 Transition of the leptotene threads into loops in the formation of the bouquet stage.

100 The typical, loose bouquet stage.

101 Synizesis stage.

102 to 104 Stages in the breaking up of the synaptic threads to form the faintly staining, anastomosing chromatin masses, characteristic of later stages.

105 Typical egg which has passed through the preceding stages and is rapidly increasing in size. Chromatin nuclei are usually very irregular and woolly.

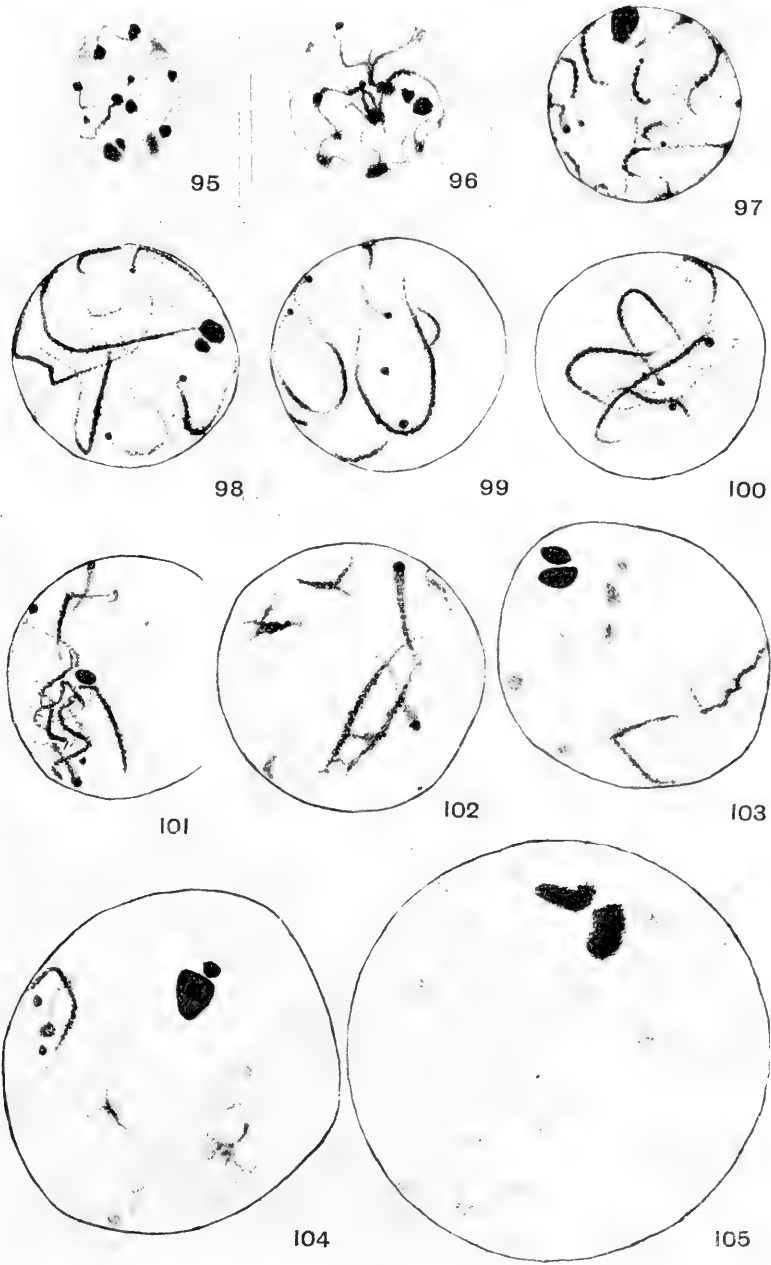


PLATE 9

EXPLANATION OF FIGURES

Spermatogonial chromosomes of other species.

106 Splitting of the metaphase chromosomes—*punctulata*.

107 Typical anaphase—*punctulata*.

108 and 109 Metaphase showing the usual twenty-two chromosomes—*punctulata*.

110 and 111 Metaphase—*ancocisconensis*.

112 and 113 Metaphase—*vulgaris*.

114 Metaphase—*purpurea*.

Abnormal mitoses

115 A spindle containing only one large chromatin element.

116, 117, and 118 An interesting arrangement of three abnormal spindles.
Figure 118 stands perpendicular to the plane of figures 116 and 117.



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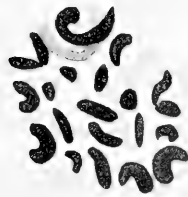
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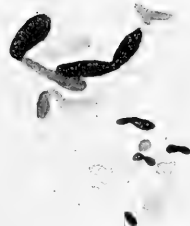
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PLATE 10

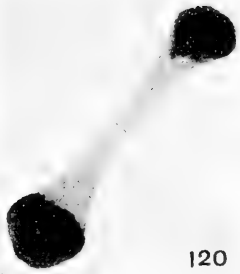
EXPLANATION OF FIGURES

Abnormal mitoses—*Continued*

- 119 A multiple chromosome group.
- 120 Telophase more than twice the normal size.
- 121, 122, and 125 Abnormal spindles being absorbed in the cytoplasm.
- 124 Anaphase with an excessive amount of chromatin.
- 123, 126, and 127 Typical multipolar spindles.



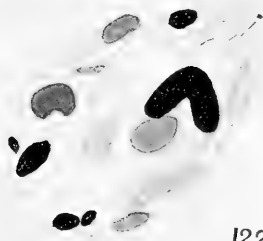
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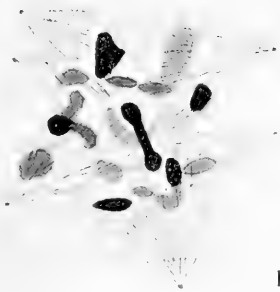
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127

Resumen por el autor, Bennet M. Allen.
Universidad de Kansas.

Desarrollo de las glándulas tiroides de Bufo y su relación normal con la metamorfosis.

La acumulación de material coloide en las glándulas tiroides de los renacuajos de sapo coincide con la aparición de los rudimentos de los miembros posteriores. Las masas coloides aumentan en tamaño y número hasta que los miembros anteriores perforan la piel. Esta acumulación de material coloide está acompañada de un marcado aumento de tamaño en las glándulas, el cual parece ser un resultado directo de aquella. El hecho aparentemente paradójico de la cesación de crecimiento y actual disminución de tamaño de las glándulas tiroides y de las masas coloides en el momento en que el proceso de la metamorfosis es mas activo, podría explicarse en parte como el resultado de un proceso parcial de desecación debido a la emergencia de los renacuajos fuera del agua, si la salida de estos fuera de dicho medio no tuviese lugar en un estado ulterior. La reducción de tamaño está pues, realizándose antes de que el factor citado pueda ser efectivo. Es mucho mas probable que tal disminución se deba a la absorción por la sangre de una cantidad considerable del coloide almacenado en las glándulas, en el momento en que dicho material puede producir mas efectos. La cola aumenta continuamente de tamaño hasta un cierto momento, presenta una ligera, disminución y desaparece después rápidamente. La secreción tiroidea no es causa de la disminución de tamaño de la cola ni alcanza un volumen considerable antes de ser suficiente para producir tal resultado. Es indudable que el desarrollo de los miembros y el proceso de desaparición de la cola siguen a la acumulación de coloide en las glándulas tiroideas de Bufo.

THE DEVELOPMENT OF THE THYROID GLANDS OF BUFO AND THEIR NORMAL RELATION TO METAMORPHOSIS¹

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ONE PLATE (SIX FIGURES) AND ONE TEXT FIGURE

In recent years much light has been thrown upon the influence of the thyroid gland upon growth. Most striking are recent studies upon the influence that this gland exerts upon metamorphosis in the amphibians. Gudernatsch ('12) showed by experimental means that feeding thyroid preparations of sheep to tadpoles of *Rana* greatly accelerated their metamorphosis. This experiment has been repeated by others (Swingle, '18) and completely verified. In 1916 the writer was successful in removing the thyroid-gland anlagen from young tadpoles, which were then reared to a period long after that at which metamorphosis should normally occur, one tadpole being kept alive until fourteen months after the operation. In all successful cases there was total failure to metamorphose, although the hind limbs underwent a limited amount of growth and the fore limbs began to develop without, however, breaking through the skin.

Hoskins and Morris ('16) successfully accomplished the removal of the thyroid gland in a similar way at the same time in *Rana* and *Amblystoma*. They had some difficulty in rearing the operated tadpoles, but were successful the following season. Their work was reported at the December, 1916, meeting of the American Association of Anatomists and again at the December, 1917, meeting.

In the spring of 1917 the writer repeated his earlier experiments upon *Rana* and also performed similar operations upon

¹Contribution No. 318 from the Department of Zoology, University of Kansas.

Bufo. These experiments clearly demonstrate that the thyroid glands exert a marked influence upon development—an excess of thyroid (feeding) accelerating metamorphosis and the removal of the thyroid gland producing decided retardation of metamorphosis. It is thus seen that these two methods of investigation give results that are fully corroborative of one another.

There are certain points in the solution of this problem that can be best attacked by a study of the normal relation of these glands to metamorphosis. It was shown in *Rana pipiens* that the thyroidless and normal control tadpoles show no differences up to the time when the limb buds begin to develop, but as soon as these have made their appearance in the thyroidless tadpoles they show a marked retardation in growth, while the body continues to grow in size. As far as our observations go, it was found that all somatic features of tadpole development were thus retarded, the gonads alone being unaffected (Allen, Rogers and Terry, '18). In order properly to interpret investigations of this kind, it is necessary to have definite data regarding the normal relation of the thyroid glands to development.

The development of the thyroid glands of Anurans was described by Goette in Bombinator. A paper by W. Müller gave the first complete account of the process. This was worked out in *Rana temporaria*. Müller found that the thyroid developed from a ventral downgrowth of the floor of the pharynx. This anlage was at first unpaired, but later became divided into two parts by the development of the copula of the hyoid cartilage. He traced the further development of the gland from a condition of a solid mass to a looser texture, accompanied by the disappearance of the pigment cells characteristic of the early stages, and he found later that it consists of a network of twisted cords which are surrounded by looser connective tissue. He traced the development through later stages in which vesicles had developed. These were composed of a single layer of epithelium and filled with colloid. He found that in young frogs immediately after metamorphosis, the thyroid gland is wholly made up of the vesicles containing colloid.

De Meuron ('86) also described the early stages in the development of the Anuran thyroid glands.

Maurer ('88) worked out the development of the thyroid gland in *Rana esculenta*. He found the division of the median anlage to take place in the 13-mm. larva. At this time the cells were deeply pigmented, loosely arranged, and showed the first beginnings of vesicle formation. His account of the further development did not take it up in great detail, but showed that by the time of metamorphosis the thyroid was well developed, being composed of a large number of follicles containing colloid. This was very clearly illustrated in one of his beautiful figures. We thus see that the general development of the thyroid has been pretty thoroughly worked out. It remains, however, to show the relation between thyroid development and the general body features which become modified during metamorphosis. A study of this kind involves close attention to the length of body, length of tail, length of limb, etc., and a comparison of these features with the volume of the thyroid at various stages of development. In order to have any force in showing the relation of the thyroid gland to metamorphosis, this work must be done in a roughly quantitative fashion. None of the investigators up to the present time have attacked the problem from this angle with one exception. Leo Adler ('14) made a few observations upon the size of the thyroid gland in different stages of *Rana temporaria*. His series was made up of one each, the length of the thyroid gland being given in parenthesis after the total length dimensions of each stage. His measurements were as follows: 20 mm. (0.07 mm.); 23 mm. (0.09 mm.); 25 mm. (0.10 mm.); 28 mm. (0.16 mm.); 30 mm. (0.21 mm.); 33 mm. (0.32 mm.); 35 mm. (0.28 mm.); 40 mm. (0.24 mm.). He states that the 28-mm. tadpoles have hind legs which show a division into joints, but no statement is made as to their length. The 33-mm. tadpoles have completely developed hind legs, while the fore legs are visible through the skin. Adler states that the last two tadpoles (35 mm. and 40 mm.) are abnormal in size. They had been hindered in metamorphosis, at first by temperature that was too high and, later, by temperature that was too low.

MATERIAL AND METHODS

A very complete collection of tadpoles of *Bufo lentiginosus* gathered in Lawrence, Kansas, afforded all of the stages, from the first appearance of the hind-limb buds to the completion of metamorphosis. A number of specimens were fixed in Flemming's fluid and others in bichromate acetic. Large quantities of *Bufo* material fixed in 5 per cent formalin were used for a study of the gross features of the development of the thyroid gland. These proved to be most valuable.

I wish to express my grateful acknowledgment of facilities and assistance accorded me by the Department of Anatomy of the University of Illinois Medical School. The greater part of the sections and illustrations used in this work were made by their technicians and artists. A series of gross dissections of *Bufo* were made in our University of Kansas Zoological Laboratory.

The sections were cut at a thickness of 10μ and were for the most part stained with haematoxylin and eosin. In some cases Heidenhain's iron alum-haematoxylin was used.

The dissections were made under a binocular microscope in such a fashion that the exposed thyroid glands were left adherent to the hyoid cartilage, the whole being stained with alum-cochineal. These dissections were dehydrated and then cleared in oil of wintergreen. They were preserved and finally studied in this fluid.

Measurements were made by means of a micrometer eyepiece, the maximum length, breadth, and thickness being determined in each case. In making a measurement of the thickness, the hyoid cartilage was held vertical between two small pieces of glass and the extreme thickness was measured in optical section. The accompanying table 1 gives the measurements obtained from a study of the dissections just mentioned. Length, breadth, and thickness of the gland were multiplied together to give a rough approximation of the volume, in effect the volume of a parallelepiped that would contain the gland. The latter is flattened oval, somewhat irregular in some instances, but for the most part of relatively constant shape.

TABLE 1

Table of measurements of Bufo lentiginosus larvae and newly metamorphosed individuals

NUMBER	TOTAL LENGTH	BODY LENGTH	TAIL LENGTH	HIND LEG LENGTH	THYREOID			VOLUME	AVERAGE VOLUME
					Length	Breadth	Thick-ness		
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	cm.	mm.
I a	21.6	9.7	11.9	1.386	R. 0.273	0.119	0.091	0.0029	0.0030
					L. 0.322	0.112	0.091	0.0032	
I b	21.1	9.6	11.5	1.320	R. 0.217	0.112	0.077	0.0017	0.0017
					L. 0.189	0.112	0.084	0.0017	
I c	20.9	9.9	11.0	1.221	R. 0.280	0.112	0.049	0.0015	0.0014
					L. 0.322	0.091	0.049	0.0014	
I d	20.1	9.6	10.5	1.353	R. 0.385	0.147	0.084	0.0045	0.0037
					L. 0.315	0.119	0.077	0.0030	
I e	22.2	9.9	12.3	1.386	R. 0.294	0.119	0.049	0.0017	0.0019
					L. 0.294	0.126	0.063	0.0021	
I f	21.1	9.9	11.2	1.188	R. 0.301	0.133	0.049	0.0019	0.0018
					L. 0.266	0.112	0.063	0.0018	
I g	20.8	10.0	10.8	1.254	R. 0.308	0.126	0.035	0.0012	0.0010
					L. 0.280	0.091	0.035	0.0008	
I h	20.1	9.7	10.4	1.056	R. 0.161	0.105	0.1028	0.0017	0.0013
					L. 0.203	0.119	0.035	0.0008	
I i	19.5	9.1	10.4	0.924	R. 0.182	0.077	0.035	0.0005	0.0005
					L. 0.210	0.084	0.028	0.0005	
I j	20.4	9.5	10.9	1.056	R. 0.245	0.091	0.042	0.0009	0.0012
					L. 0.294	0.133	0.035	0.0015	
II a	26.2	11.8	14.4	4.521	R. 0.399	0.231	0.154	0.0138	0.0146
					L. 0.483	0.182	0.182	0.0155	
II b	26.1	11.9	14.2	2.739	R. 0.378	0.203	0.133	0.0099	0.0109
					L. 0.420	0.224	0.126	0.012	
II c	25.0	11.1	13.9	4.026	R. 0.378	0.231	0.105	0.0087	0.0090
					L. 0.336	0.231	0.119	0.0094	
II d	26.0	11.9	14.1	2.574	R. 0.385	0.217	0.140	0.0117	0.0113
					L. 0.364	0.231	0.119	0.0109	

TABLE 1—Continued

NUMBER	TOTAL LENGTH	BODY LENGTH	TAIL LENGTH	HIND LEG LENGTH	THYROID			VOLUME	AVERAGE VOLUME
					Length	Breadth	Thick-ness		
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>cm.</i>	<i>mm.</i>
II e	25.8	12.1	13.7	2.673	R. 0.315	0.182	0.112	0.0061	0.0072
					L. 0.385	0.196	0.112	0.0084	
II f	23.9	11.5	12.4	3.432	R. 0.378	0.217	0.133	0.0108	0.0097
					L. 0.392	0.196	0.112	0.0086	
II g	26.2	12.8	13.4	4.389	R. 0.336	0.210	0.147	0.0107	0.0115
					L. 0.315	0.224	0.182	0.0123	
II h	25.2	11.0	14.2	4.818	R. 0.364	0.252	0.168	0.0153	0.0258
					L. 0.651	0.315	0.182	0.0363	
II i	25.1	11.2	13.9	2.640	R. 0.420	0.231	0.133	0.0102	0.0088
					L. 0.378	0.161	0.122	0.0074	
II j	25.8	11.2	14.6	4.884	R. 0.385	0.203	0.161	0.0123	0.0172
					L. 0.497	0.266	0.175	0.0222	
III a	27.2	12.2	14.0	7.227	R. 0.504	0.308	0.206	0.0325	0.0261
					L. 0.434	0.266	0.168	0.0197	
III b	25.7	12.4	13.3	7.326	R. 0.525	0.315	0.217	0.0355	0.300
					L. 0.511	0.301	0.161	0.0245	
III c	26.0	12.1	13.9	7.194	R. 0.462	0.273	0.175	0.0211	0.230
					L. 0.455	0.287	0.189	0.0248	
III d	27.2	12.2	15.0	7.194	R. 0.455	0.301	0.189	0.0256	0.0232
					L. 0.406	0.301	0.168	0.0209	
III e	27.1	12.4	14.7	8.283	R. 0.413	0.301	0.182	0.0211	0.0233
					L. 0.455	0.308	0.189	0.0256	
III f	26.9	11.2	15.7	8.283	R. 0.427	0.294	0.196	0.0249	0.0302
					L. 0.511	0.294	0.245	0.0355	
III g	27.1	12.4	14.7	7.161	R. 0.476	0.322	0.182	0.0276	0.0276
					L. 0.441	0.329	0.189	0.0276	
III h	28.1	12.2	15.9	7.524	R. 0.420	0.294	0.182	0.0219	0.0194
					L. 0.399	0.252	0.175	0.0170	
III i	27.8	11.9	15.9	8.679	R. 0.518	0.350	0.224	0.0400	0.0221
					L. 0.238	0.154	0.119	0.0043	

TABLE 1—Continued

NUMBER	TOTAL LENGTH	BODY LENGTH	TAIL LENGTH	HIND LEG LENGTH	THYROID			VOLUME	AVERAGE VOLUME
					Length	Breadth	Thick-ness		
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	cm.	mm.
III j	27.1	11.7	15.4	8.745	R. 0.427 L. 0.409	0.329 0.357	0.259 0.238	0.0369 0.0354	0.0361
IV a	23.1	10.9	12.2	9.636	R. { 0.392 0.280 L. 0.371	0.308 0.147 0.350	0.206 0.210	0.053 0.272	0.0263
IV b	25.3	10.5	14.8	9.702	R. 0.385 L. 0.392	0.273 0.266	0.154 0.133	0.0154 0.0137	0.0145
IV c	23.6	10.6	13.0	8.943	R. 0.413 L. 0.427	0.259 0.259	0.168 0.168	0.0181 0.0190	0.0185
IV d	25.4	11.3	14.1	9.306	R. 0.574 L. 0.623	0.343 0.322	0.210 0.206	0.0407 0.0418	0.0412
IV e	26.8	11.9	14.9	9.966	R. 0.567 L. 0.609	0.364 0.385	0.210 0.206	0.0433 0.0487	0.0460
IV f	26.5	12.0	14.5	8.778	R. 0.497 L. 0.525	0.322 0.343	0.210 0.224	0.0336 0.0389	0.0362
IV g	25.0	11.3	13.7	8.943	R. 0.518 L. 0.525	0.329 0.266	0.175 0.154	0.0292 0.0211	0.0251
IV h	25.6	11.5	14.1	10.131	R. 0.504 L. 0.490	0.357 0.315	0.217 0.175	0.0396 0.0258	0.0327
IV i	24.6	10.4	14.2	8.844	R. 0.532 L. 0.490	0.322 0.315	0.208 0.231	0.0406 0.0349	0.0377
IV j	21.7	10.9	10.8	7.448	R. 0.511 L. 0.560	0.315 0.336	0.206 0.217	0.0332 0.0419	0.0375
V a	12.9	11.6	1.3	10.956	R. 0.385 L. 0.413	0.329 0.301	0.182 0.210	0.0226 0.0258	0.0242
V b	14.3	11.0	3.3	10.560	R. 0.497 L. 0.413	0.364 0.413	0.203 0.225	0.0362 0.0403	0.0381
V c	14.9	11.6	3.3	9.735	R. 0.490 L. 0.364	0.273 0.287	0.175 0.168	0.0225 0.0177	0.0201
V d	12.9	11.8	1.1	10.923	R. 0.602 L. 0.483	0.371 0.371	0.217 0.175	0.0488 0.0302	0.0395

TABLE 1—*Concluded*

NUMBER	TOTAL LENGTH	BODY LENGTH	TAIL LENGTH	HIND LEG LENGTH	THYROID			VOLUME	AVERAGE VOLUME
					Length	Breadth	Thick-ness		
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
V e	13.8	11.9	1.9	10.890	R. 0.483 L. 0.434	0.378 0.336	0.175 0.238	0.0310 0.0351	0.0480
V f	14.8	11.9	2.9	10.857	R. 0.392 L. 0.409	0.329 0.315	0.161 0.182	0.0106 0.0229	0.0167
V g	12.3	11.7	0.6	11.550	R. 0.476 L. 0.409	0.336 0.357	0.280 0.287	0.0457 0.0438	0.0442
V h	13.1	11.7	1.4	9.801	R. 0.497 L. 0.532	0.287 0.336	0.182 0.231	0.0261 0.0414	0.0337
V i	12.9	11.5	1.4	9.207	R. 0.371 L. 0.427	0.266 0.294	0.168 0.196	0.0240 0.0249	0.0244
V j	13.0	11.1	2.9	10.395	R. 0.392 L. 0.364	0.259 0.287	0.182 0.182	0.0182 0.0188	0.0185
VI a	Tail completely absorbed.	12.8	Tail completely absorbed.	10.659	R. 0.623 L. 0.567	0.406 0.413	0.368 0.287	0.0788 0.0679	0.0733
VI b		13.5		13.299	R. 0.616 L. 0.497	0.385 0.441	0.287 0.673	0.0681 0.0594	0.0637
VI c		13.8		12.419	R. 0.651 L. 0.567	0.392 0.385	0.294 0.301	0.0735 0.0650	0.0692
VI d		12.9		12.507	R. 0.413 L. 0.448	0.308 0.294	0.294 0.234	0.0368 0.0287	0.0327
VI e		12.3		11.055	R. 0.658 L. 0.497	0.448 0.455	0.294 0.294	0.0719 0.0667	0.0792
VI f		12.7		10.923	R. 0.483 L. 0.511	0.364 0.399	0.252 0.301	0.0432 0.0612	0.0522
VI g		12.3		12.474	R. 0.518 L. 0.581	0.315 0.322	0.301 0.308	0.0484 0.0520	0.0502
VI h		11.4		11.088	R. 0.462 L. 0.532	0.273 0.294	0.224 0.206	0.0273 0.0323	0.0298
VI i		11.9		11.979	R. 0.477 L. 0.448	0.322 0.308	0.294 0.301	0.0445 0.0418	0.0431
VI j		12.2		10.527	R. 0.504 L. 0.455	0.357 0.392	0.294 0.266	0.0506 0.0474	0.0490

The tadpoles selected from a jar containing many hundred were closely matched in six representative stages with ten specimens of each stage. The measurements of lengths of hind leg, body, and total length give a basis for comparing these lots. Of these criteria the most constant is the length of the hind legs. These show a continuous growth, while the total length and body length are modified by the process of metamorphosis, the body showing distinct reduction for a time. The stages chosen may be described as follows:

I. Hind-limb buds very small, the longest showing but faint indications of differentiation into parts. No evidence of fore legs (fig. 1 *a*).

II. Total length and body length increased. Hind limbs showing differentiation into parts. Toes well differentiated.

III. Hind limbs decidedly larger than in preceding stage. Continued increase in total and body length. Fore limbs formed beneath the skin, but not yet broken through (fig. 2 *a*).

IV. Continued increase in length of hind limbs. Fore limbs through the skin. Slight decrease in total length and body length.

V. Continued increase in size of limbs. Slight increase in body length, but marked decrease in tail length.

VI. Completion of metamorphosis (fig. 3 *a*).

In the main there is little need of comment upon the figures in the accompanying tables. In stage I (fig. 4) there is little colloid present in the follicles of the thyroid glands and many of the follicles are not yet formed, being represented merely by small scattered masses of cells. They lie all in one plane at this time, except in a few cases where they are beginning to arrange themselves in two layers. This process is completed in stage II, where the thyroid glands show a distinct increase in size and in the number and size of the component follicles (fig. 5). This is continued through later stages. It will be seen that there are many cases where the volume of the thyroid glands is not proportional to the relative length of the legs or of the body. While this is true in a comparison between the members of group I and group II (with the single exception of II *h*), there are no

members of the former group that have a thyroid gland volume approaching that of any member of group II. The same is true in comparing group II with group III (with the single exception of II *h*). These statements do not hold true, however, in comparing groups IV, V, and VI. In these there are a number of cases in which members of a younger stage will show a greater volume of the thyroid glands than do certain individuals in the higher groups. In fact, the average volume of the thyroid glands in group V is less than that of group IV. This point will be discussed later. Even among the metamorphosed toads, VI *h*, for instance, shows a thyroid gland volume less than the

TABLE 2

Dimensions of thyroid glands and body measurements of Bufo lentiginosus larvae

STAGE	TAIL LENGTH	TAIL PROPOR- TION	BODY LENGTH	BODY PROPOR- TION	LEG LENGTH	LEG PROPOR- TION	THYROID VOLUME	THYROID $\sqrt[3]{\text{VOLUME}}$	THYROID PROPOR- TION
	<i>mm.</i>		<i>mm.</i>		<i>mm.</i>		<i>mm.</i>		
I	11.09	0.742	9.69	0.770	1.21	0.104	0.00139	0.0518	0.0635
II	13.88	0.954	11.65	0.926	3.67	0.314	0.01270	0.5026	0.616
III	14.95	1.000	12.07	0.959	7.76	0.664	0.02612	0.6390	0.771
IV	13.63	0.912	11.13	0.885	9.17	0.785	0.03161	0.6811	0.834
V	2.91	0.195	11.58	0.920	10.49	0.898	0.03077	0.6753	0.828
VI	0.00	0.000	12.58	1.000	11.69	1.000	0.05427	0.8158	1.000

averages of groups IV and V, while the thyroid volume of VI *d* is surpassed by the thyroid volume of several individuals in each of the two preceding classes. It may be pointed out that this is partially to be explained by the fact that these three stages are really passed through in a relatively short period of time. Figure 6 shows the thyroid glands of VI *f*.

Table 2 shows the average dimensions of the body and of the thyroid gland in each of these groups. The actual dimensions are given, and in the following column is shown, in each case, the proportional size of the feature as compared with the size at the stage when it shows its maximum development.

Text figure A gives a graphic representation of these features as seen in table 2. In this case the growth of the hind limbs was taken as a standard for determining the relative stage of devel-

opment in each group. With the material at hand it was impossible to judge the age of the specimens. This would be quite an unsatisfactory method of seriating material, even in laboratory-reared specimens, because of the large amount of individual variation in the rate of growth of tadpoles. Temperature conditions play a large part in determining the rate of growth. Any attempt to regulate this factor would probably entail abnormal

Relation between thyroid growth and metamorphosis in *Bufo lentiginosus*

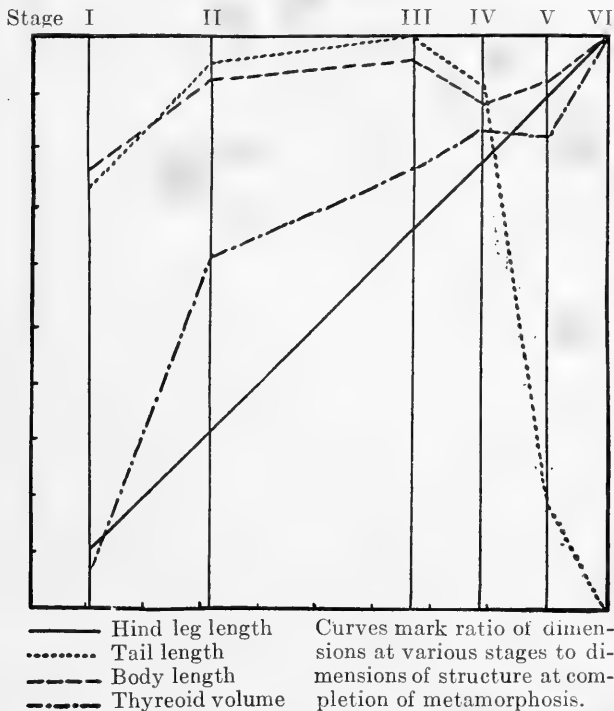


Fig. A Curves to show the relative rate of growth of the total length, body length, and hind-limb length as compared with the growth of the thyroid gland during metamorphosis in *Bufo lentiginosus*.

conditions that would modify growth in other ways. It is difficult, at best, to bring about normal development of tadpoles under laboratory conditions. For these reasons it was decided to use specimens caught under natural conditions and seriated as indicated above.

The growth of the hind limbs was arbitrarily represented by a straight line inclined at an angle of 45° . Upon this were marked off intervals indicating the average length of the hind limbs at the given stage as compared with the average length at the stage of metamorphosis (stage VI). From each of these points a perpendicular was dropped to the base line. These perpendiculars then served to indicate the six stages chosen. Their distance from each other serving to indicate the probable time intervals between the different stages upon the assumption that the growth of the hind limbs takes place at a uniform rate. Points established upon these verticals serve to indicate the average dimensions of various features at each of the six stages studied, the height from the base line showing the proportion that the dimensions of any given feature of that stage bear to its dimensions at the time of metamorphosis—stage VI—maximum development in the case of tail length. Curves were constructed by joining these points, thus giving the proportional rate of growth of each feature. The cube root of the volume of the thyroid gland was employed because it would represent one dimension of a cubical figure whose volume would roughly represent the volume of the thyroid gland. This appeared to be the best criterion of comparison, because each of the other features was represented by a one-dimension value as body length, tail length, and hind leg length. In reality all of these features have length, breadth, and thickness. Any influence that the thyroid gland would exert upon their growth would be the influence of one solid body upon another. The length of the thyroid gland could not be taken as a criterion of comparison, because it increases little during the stages, while the volume of the gland increases greatly, owing to growth in thickness. Thus it seems that the fairest basis of comparison would be to compare the cube root of the volume of the thyroid with the length dimensions of the body, tail, and hind limb.

It is noted that the cube root of the thyroid volume shows a marked rise from stage I to stage II, from which the rise continues strongly to stage III, then more strongly to stage IV. There is a slight fall in the curve from stage IV to stage V, with

a sharp rise to stage VI. The body length shows a steady rise to stage III, when it falls off quite distinctly. This is probably due to the shrinkage of the intestine which brings the cloacal opening closer to the root of the tail than it had previously been. It is just at this time that the fore limbs have first appeared. The partly metamorphosed toads are leaving the water at stage V, and an appreciable loss of water from the tissues must take place. However, the body has really begun again to increase in size at stage V, and by stage VI it has exceeded the length attained in stage III. The tail reaches its maximum length in stage III, and then rapidly diminishes to the vanishing point.

The cube root of the thyroid gland increases more rapidly than does the length of the hind legs during the interval between the first and second stages. This is significant in that it corresponds with the results of experimental work which show that the hind limbs develop very slowly in tadpoles from which the thyroid glands have been extirpated. It is thus seen that growth of the hind legs is to a very large extent dependent upon the growth of the thyroid gland.

A study of sections of the thyroid glands shows that colloid begins to form at about the time when the hind limbs commence to develop. Compare figures 1 *b*, 2 *b*, and 3 *b* and table 3. It increases in amount as growth continues. Measurements of the diameter of the larger colloid masses at different stages of development show a steady increase in size, very rapid in the early stages, as seen in table 3. In each specimen micrometer measurements were made of ten of the larger colloid masses. An average was then calculated in each case (table 3).

The colloid masses increase in size until stage III, while in V there is a diminution in size. This is observed even in some of the metamorphosed toads, while in others the colloid masses have reached a size beyond that found in stage III. This table is clearly based upon too few observations to prove in itself of much value. It is of significance, however, in that it corresponds in a general way with the results of table 1. We see that the increase in size of the thyroid gland corresponds with an increase in size of the colloid masses. It appears that this

growth of the glands is, in fact, brought about by the accumulation of colloid substance. In the first stages of colloid formation there were only from five to ten colloid masses. These soon became very numerous, as shown in figures 1 *b*, 2 *b*, and 3 *b*. No satisfactory conclusions were drawn from a study of the epithelium of the follicles, although it is quite possible that important points might be gained by an application of special methods of technique upon the problem of the manner in which they elaborate the thyroid secretion.

TABLE 3

Table of measurements of colloid masses in the thyroid gland of Bufo lentiginosus

TOTAL LENGTH	BODY LENGTH	HIND-LEG LENGTH	AVERAGE COLLOID DIAMETER
<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
8.4	3.4		No colloid present
8.4	3.7		
8.9	3.8		
9.1	4.3		
11.4	5.5	0.245	0.0122
14.6	6.9	0.357	0.0138
15.1	8.4		0.0147
20.8	9.5		0.0284
20.8	10.6	2.805	0.0258
23.6	11.8	4.884	0.0559
24.8	11.4	6.270	0.0603
13.1	10.1	7.623	0.0499
12.0	12.0	10.164	0.0478
12.3	12.3	11.055	0.0672

SUMMARY AND CONCLUSIONS

The accumulation of colloid material in the thyroid glands of toad tadpoles begins just as the hind limb buds appear. The colloid masses continue to increase in size and number until the fore limbs break through the skin. This accumulation of colloid material is accompanied by a marked increase in the size of the thyroid glands, which appears in the main to be a direct result of it.

A series of observations upon the effect of thyroid extirpation in *Rana* and *Bufo* upon limb development have shown that the

limb buds appear simultaneously in both the control and operated tadpoles. Soon after their appearance the limb buds of the thyroidless tadpoles lag far behind those of the normal controls. They finally grow to an appreciable degree in spite of the absence of the thyroid gland, but never so fast nor to any degree approaching the length relative to body length attained in the normal controls. These observations will be extended during the coming season and published later. It is clear, however, that the effects of thyroid removal first become evident in Bufo at the period when colloid normally begins to accumulate. A comparative study along this line would give some valuable hints upon the real significance of colloid secretion and accumulation.

We have next to consider the apparently paradoxical fact that there is a cessation in growth and an actual diminution in the size of the thyroid glands and of the colloid masses at the very time when the process of metamorphosis is most active (stage V). This might in part be explained as the result of a partial drying process due to the emergence of the tadpoles from the water, were it not for the fact that they do not emerge upon the land until stage V. The reduction in size is thus under way before this factor could prove effective. It is much more probable that this diminution may be due to the absorption of an unusually large amount of stored colloid at this time when it would prove most effective. It is quite conceivable that substances might be elaborated in the blood that would enable it to more readily dissolve the colloid and that its solvent power might decrease again after metamorphosis has been completed. Of course this is pure conjecture, but it is put forth in the hope that it may prove suggestive.

The development of the tail presents an interesting problem. It steadily increases in size until stage III, shows a slight diminution to stage IV, and then quickly disappears. It might be assumed that a certain amount of thyroid secretion must be elaborated before the absorption of the tail can be accomplished, or, if our assumption of a more solvent condition of the blood should prove true, it might serve to explain this point. What-

ever the means by which it is accomplished, we should have to choose between two alternatives: either the thyroid secretion does not cause the shrinkage of the tail, or it must reach a considerable volume before it is able to accomplish that result. It is certain that limb development and the process of disappearance of the tail follow the accumulation of colloid in the thyroid glands of *Bufo*.

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PLATE

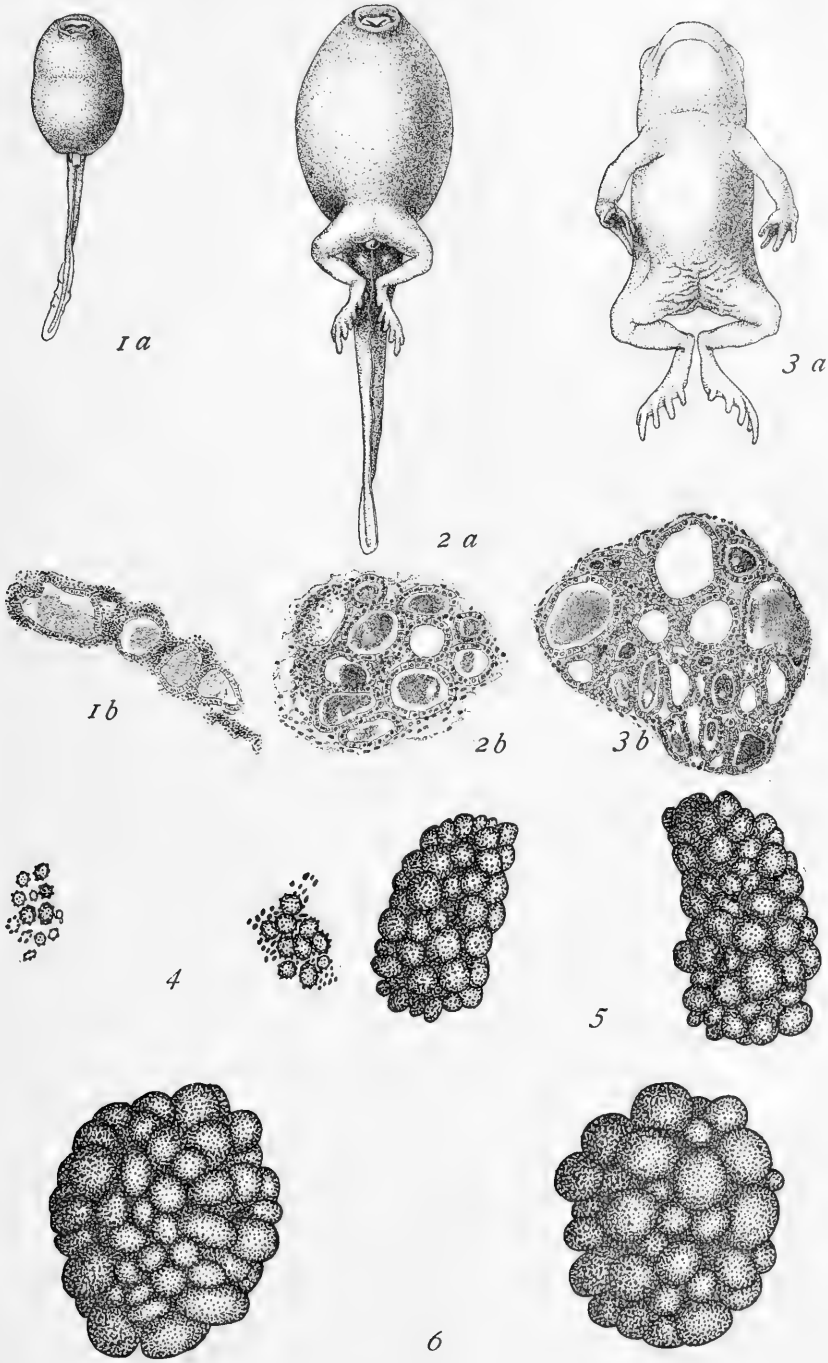
PLATE 1

EXPLANATION OF FIGURES

1a, 2a, and 3a Drawings to show typical stages in the development of *Bufo lentiginosus*. Figure 1a represents stage I. Figure 2a represents stage III. Figure 3a represents stage VI. All drawn to scale and magnified $\times 3$.

1b, 2b, and 3b Drawings to show transverse sections of the thyroid glands of 1a, 2a, and 3a, respectively. Figure 1b, transverse section of the thyroid gland of 1a. Figure 2b, transverse section of the thyroid gland of 2a. Figure 3b, transverse section of the thyroid gland of 3a. All drawn to scale and magnified $\times 75$.

4, 5, and 6 Whole mounts of the thyroid glands of the stages represented above. Figure 4, whole mount of thyroids of I b. Figure 5, whole mount of thyroids of II h. These thyroids have a volume almost identical with the average in group III. Figure 6, whole mount of thyroids of VI f. All drawn to scale and magnified.



Resumen por el autor, Waro Nakahara.
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Estudio de los cromosomas en la espermatogénesis de *Perla immarginata* Say, con especial mención del problema de la sinapsis.

El presente trabajo es un estudio del elemento cromático en la espermatogénesis de *Perla*, hasta el final de la segunda división de los espermatocitos. En el complejo espermatogonial existen diez cromosomas (incluyendo los cromosomas X e Y). El espermatocito de primer orden posee cinco cromosomas bivalentes; los cromosomas X e Y están fusionados entre sí y aparecen como un solo elemento. En el espermatocito de segundo orden existen cinco cromosomas univalentes; cada espermatocito recibe uno de los cromosomas X e Y. El autor discute el modo de formación de los cromosomas bivalentes. En *Perla* los cromosomas del complejo espermatogonial forman parejas. Los cromosomas homólogos se unen por telosinapsis en el espirema del espermatocito de primer orden, y mas tarde se incurvan uno hacia el otro en el punto sináptico para formar anillos y tetradas antes de la metafase de la división. No hay pruebas sobre la parasinapsis en un estado temprano de la división.

Translation by José F. Nonidez
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A STUDY ON THE CHROMOSOMES IN THE SPERMATOGENESIS OF THE STONEFLY, *PERLA IMMARGINATA* SAY, WITH SPECIAL REFERENCE TO THE QUESTION OF SYNAPSIS

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THREE PLATES (FIFTY-ONE FIGURES)

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INTRODUCTION

The need of accurate knowledge of the chromosomes, and especially their behavior in the maturation divisions, can hardly be overemphasized. The chromosome theory of inheritance, so beautiful a unification of biological knowledge as it is, cannot be fully established until the true nature of synapsis and other phenomena involved in the course of maturation divisions are satisfactorily understood.

The present contribution to the study of the chromosomes is based on my observations on the spermatogenesis of the stonefly, *Perla immarginata* Say (Plecoptera). The fact that, notwithstanding recent cytological activity, the order Plecoptera has not been made a subject for chromosome study led me to make some preliminary observations on a few species of this group of insects during the fall of 1916. *Perla immarginata*,

which is one of the commonest stoneflies in Ithaca (Smith, '13), was then determined as best fitted for minute investigations, because of the favorable condition of its chromosomes. Since the spring of 1917 observation on this form has been carried on in the Laboratory of Histology and Embryology, Cornell University, under the supervision of Prof. B. F. Kingsbury, whose helpful suggestions and criticisms given to me during the course of the work are most sincerely appreciated. A prolonged observation was made with the greatest care I am capable of, and the numerous sketches made were carefully compared, and the working out of the history of the chromatin element was thus ventured.

TECHNIQUE

Flemming's strong fluid has proved to be the best for fixing the testis dissected out in normal salt solution. Bouin's picro-aceto-formol has also been used very frequently. Notwithstanding the powerful penetration of the fluid, the result was no better than that obtained from Flemming's fluid. Bouin's fluid tends to make the split line of the spireme more or less obscure, and the material of this fixation was thus found to be unfavorable for observations of certain critical stages. As far as the penetration is concerned, even Flemming's fluid seems to be powerful enough, when applied on dissected material, to say nothing about Bouin's. Addition of a small amount of urea to Bouin's fluid (Hance, '17) did not make any noticeable change in the fixation from the original formula.

Sections were cut from 7 to 10 μ thick and stained with Heidenhain's iron hematoxylin. The longer method with this stain, resulting in black coloring of the section, was more favorable than the shorter 'blue' method, especially for the observations on the nucleus in the early prophase of mitosis.

DESCRIPTIVE

Ten chromosomes appear in the spermatogonial division (figs. 1 and 2). Looking at a metaphase plate from either pole, all of these ten can be individually recognized in every case. The

chromosome group consists of two pairs of V's, a pair of rods, two spherules (m-chromosomes), and two unpaired rods, one of which is much longer than the other. These last I interpret as X- and Y-chromosomes, respectively.

Figures 3 to 5 show the changes in the nucleus subsequent to the last spermatogonial division. The nucleus in the late anaphase of the division (fig. 3) gradually enters into the resting stage (figs. 4 and 5), when the reticular appearance of the nucleus is resumed.

The chromatin reticulum then begins to form the double spireme. The process of the formation of the double spireme is illustrated in figures 6 to 13, and may be best described as a development of dual threads out of the reticulum. There is no sign of two threads coming to conjugate side by side. It is also impossible to say that the process involves the actual splitting of a simple thread. As may be seen very clearly, there are no definitely formed and separate simple threads (i.e., chromosomes) in the nucleus before the development of the double spireme commences.

The stage of contraction seems to follow the completion of double threads. This seems to correspond to the period of synizesis of some authors. The nuclei in this condition are shown in figures 14 and 15, and it will be readily seen that the contraction has nothing to do with the formation of the double threads. The spireme at this stage is unquestionably already double in structure. More or less regularly accompanying the contraction nuclei, there are a number of nuclei in the process of degeneration. Four degenerative cells are shown in figures 16 to 18.

The duality of the spireme (zygotene thread) seems to be maintained all through the later stages (figs. 19 to 23).

The actual number of the prochromosomes could be counted at the pachytene stage (figs. 24 to 26), when the nuclei enlarge a little, and the spireme threads become more compact. As can be seen in figures 24 to 34, there are six separate segments recognizable at this stage. The smallest one, which is sometimes attached to the largest (fig. 32), will be seen to represent the

m-chromosome. Two other small segments, which are often seen joined together (fig. 26), can be interpreted as representing the X- and Y-chromosomes.

Excepting the X- and Y-segments (and the m-segment, of which nothing definite was observed), halves of each segment bend toward each other, until they come to lie closely side by side. This interpretation of the process of tetrad formation may receive justification through a comparison of figures 24 to 33.

The tetrads thus formed now arrange themselves on the equatorial plate of the spindle (fig. 33). Polar view of the plate shows five chromosomes as distinct bodies (figs. 35 to 37). The X and Y are joined to each other and appear as a single body. Of the remaining four, one is decidedly smaller than the others and undoubtedly identifiable with the m-chromosome. Three others, although they vary more or less in their appearances, may therefore be considered as representing the three pairs of the diploid chromosomes (two pairs of V's and a pair of rods in the spermatogonial group). Looking from the side, it will be seen that the X and Y separate with the division of the bivalents (figs. 34, 38 to 40). Figures 41 to 44 show the anaphase of the first division and the prophase of the second, in which there is no resting stage.

If the process of ring and tetrad formation be that of an opening out of a split chromosome, as it is frequently interpreted, the space enclosed by a ring must correspond to the longitudinal split in the zygotene thread. That this interpretation does not hold in the case of *Perla* may easily be seen from a comparison of figures 21, 27, 33, and 34. The longitudinal split of the zygotene spireme persists as such in the ring and even in the chromosome on the metaphase plate, and may be best interpreted as a precocious split for the second spermatocytic division, which follows the first division without the 'resting stage.' It has nothing to do with the space enclosed by a ring, which is secondarily formed when the two arms of a bivalent segment become joined.

The chromosome number in the second spermatocytic division is five. The five consist of two V's, one rod, 'm', and X- or Y-chromosome (fig. 46). From what has been observed in the preceding division, it is to be expected that half the number of the second spermatocytes should contain one accessory chromosome, and the other half of them the other, and this is apparently what takes place here.

All five chromosomes divide equationally in the second spermatocytic division, neither of the accessories is heterotropic (figs. 44 and 45).

The anaphase of the division (figs. 48 and 49) is immediately followed by the formation of the spermatids (figs. 50 and 51).

The further history of the spermatids has not been followed.

GENERAL CONSIDERATIONS

Pairing of chromosomes and probability of synapsis

The idea of the paired association of chromosomes was first suggested by Sutton ('02), when he noted in *Brachystola* that all chromosomes could be associated into pairs according to the size characters. More data were accumulated later from both zoological and botanical sides, and there seems to be no doubt at present that, where chromosomes of different sizes and shapes are present, there are always two of each kind (excepting accessory chromosomes). In his extensive work on the topic, Metz ('16) has shown with special clearness in about eighty species of *Diptera* which he examined, that the chromosomes are uniformly associated in pairs in diploid cells, in all tissues, somatic as well as germinal, and in all stages of ontogeny (from egg to adult), and he stated that pairing chromosomes give an actual demonstration of a side-by-side approximation of corresponding chromosomes.

The probability of synapsis becomes stronger, when inquiries are made as to the nature of the pairing. In a certain Hemipteron, Wilson ('09) has described, beside the regular coupling of idiochromosomes of unequal sizes, that a small supernumerary chromosome which is indistinguishable from the m-chromosome

always couples with the much larger idiochromosome, but never with the m-chromosome, and suggested that the coupling results from definite affinities among the chromosomes. He said:

The possibility no doubt exists that the couplings are produced by extrinsic cause (such as the achromatic structure), but the evidence seems on the whole opposed to such a conclusion. I consider it more probable that they are due to intrinsic qualities of the chromosomes and that the differences of behavior shown by different forms may probably be regarded as due to corresponding physico-chemical differences

Very similar statements were made by Metz ('16), who said:

Pairing (of chromosomes) is not due to purely mechanical causes, but is dependent in some way upon the qualitative nature of the chromosomes. This conclusion seems evident from the fact that paired chromosomes are corresponding or similar chromosomes. It is difficult to conceive how purely mechanical forces can cause anything more than random pairing, while as a matter of fact the actual pairing is selective to the highest degree. That this association is not merely assortment according to size is shown by the pairing of unequal sex-chromosomes in the male, where X is several times as large as Y.

Metz said further that the paired chromosomes are qualitatively similar and "their association is dependent upon, although not necessarily caused by, this relation." Convincingly supporting this statement, he pointed out that in the tetraploid groups in *Diptera*,

two of the four chromosomes are sister halves of the other two, and hence are respectively similar to them in make up. But all four of these chromosomes associate in essentially the same manner, i.e., paired chromosomes are indistinguishable from sister chromosomes in their manner of association.

Turning our attention to the case of *Perla*, we see at once that

1. The ten chromosomes in the spermatogonial group may be grouped in pairs, and that

2. Each of the pairs is represented by a single chromosome of corresponding appearance in the spermatocytic groups (excepting the X- and Y-chromosomes).

The X- and Y-chromosomes are seen actually coupling in the late prophase and in the metaphase of the first spermatocytic

division. The fact that these two chromosomes are of totally different sizes and shapes, and that the coupling takes place most regularly between these two, seems to signify much, because these afford a complete demonstration of the occurrence of synapsis, in so far as these two chromosomes are concerned. Although this does not show that a similar process must take place in other pairs of chromosomes, it does, nevertheless, add more to the probability of the general occurrence of synapsis. However, it is evident that the case of the ordinary chromosomes must be established through their direct study, for it is not without reason to suspect that some differences may be found in the process from that seen in the case of the accessory chromosomes.

Suggested modes of synapsis

In the case of Perla, facts show for certain of the chromosomes, and hence with probability for all the other chromosomes, that synapsis does take place in the maturation of the germ cells. Before entering into the closer examination of the critical stages where the process of synapsis may possibly be involved, it would be well to review briefly the interpretations of some of the previous authors.

Vejdowsky ('07) is of the opinion that the chromosomes in normal number conjugate parasynaptically and fuse completely. The mixochromosomes (haploid number) thus produced split longitudinally at both divisions.

Bonnevie ('07, '08 a, '08 b, '11) considers that the diploid chromosomes conjugate parasynaptically, and although the conjugants fuse completely in the maturation period, during which they do not separate, they ultimately become distinct.

According to Henking ('90-'92) and Korschelt ('95), the spireme segments into the diploid number of chromosomes, all of which undergo longitudinal splitting and remain separate until the metaphase, when they conjugate and appear again in the haploid number. The conjugants may separate at the first division. Goldschmidt ('08 a, '08 b) is of the same opinion, but he maintains that the separation of the conjugants takes place at the second division.

Rückert ('92, '93), Haecker ('95), and vom Rath ('95) believe that the spireme first splits longitudinally and later segments into the haploid number of the chromosomes. Each segment then undergoes transverse segmentation. At the first maturation division the separation takes place along the longitudinal splitting which first appeared and the transverse division is effected at the second division.

Paulmier ('98, '99) and Foot and Strobell ('05) consider that the haploid chromosomes are produced by telosynapsis of the diploids and later they split longitudinally. The first division takes place along the line of the conjugation and the second one along the line of the splitting.

In the views of Montgomery ('04), Farmer and Moore ('05), Mottier ('05, '07, '09), Schaffner ('07), Gates ('08, '09, '10), Yamanouchi ('09), Farmer and Schove ('14), and Nothnagel ('16), the spireme segments into a haploid number of loops, each loop consisting of two chromosomes united end to end. These later bend to a side-by-side position and separate at the first maturation division. The duality of the spireme thread before it segments into loops is regarded as a precocious split for the second division.

According to Winiwarter ('00), Gregoire and Wygart ('03), Gregoire ('04, '10), Berghs ('04), Schreiners ('06 a, '06 b), Rosenberg ('04, '08), Overton, ('05, '09), Allen ('05), Miyake ('05), Tischler ('05), Strasburger ('05, '08, '09), Janssens ('05, '09), Yamanouchi ('08), Montgomery ('11), Stevens ('12), Wilson ('12), Kornhauser ('14, '15), Robertson ('16), and Wenrich ('16, '17), diploid chromosomes conjugate parasynaptically early at the leptozygotene stage. The spireme segments into the haploid number of pieces, each of these opens out along the line of the original conjugation, and the conjugants finally separate at the first maturation division.

The citations given cover only a small part of the entire literature relating to the topic, but they nevertheless represent the several modes of synapsis that have been suggested, and include only those that are based upon comparatively accurate observations.

A glance at the above review may suffice to reveal that there are two fundamentally different views regarding the modes of synapsis—those of the parasynaptist and of the telosynaptist.

The opinions of the telosynaptists have been strenuously opposed by von Winiwarter, Gregoire, Janssens, and others, because earlier authors of this school have more or less entirely overlooked a certain stage in the early prophase, which parasynaptists claim as supporting their views. The fact that this critical stage is observable only with difficulty may well add to the dignity of the teaching of the parasynaptists, although the very same fact may also let one doubt as to the reality of the conception.

There is no doubt that the majority of cytologists to-day feel quite justified in accepting the universal occurrence of parasynapsis, probably partly due to the development of the chromosome theory of inheritance. It is significant, therefore, that in spite of the overwhelming number of the parasynaptists, there are a few who still insist upon the truth of telosynapsis.

Arnold ('09), for instance, concisely discussing Planarian spermatogenesis, concludes that "the spireme is gradually elaborated out of a reticulum and is in the earliest stage in which it can be recognized as spireme, composed of several separate segments," which are in haploid number, and never do the segments in leptotene nuclei pair up longitudinally.

One of the best botanical works supporting telosynapsis is that of Nothnagel ('16). He asserted that in *Allium* the double thread in the premeiotic nucleus is due to the splitting of the single thread, by means of essentially the same process as in somatic mitosis, and each segment appearing in the haploid number represents two diploid chromosomes united end to end.

Some seem to believe that both para- and telosynapsis may take place for different chromosomes in the same cell. Gates ('11) tries to show that the modes of synapsis may differ according to the sizes of the chromosomes. Wilson ('12) seems to admit parasynapsis for autosomes, describing at the same time an actual telosynapsis for accessory chromosomes. Payne ('14), finally, describes two different methods of ring formation in the

first spermatocytic division in Forficula, namely, by bending of a rod and by opening up of a longitudinally split thread.

Critical points

In the entire history of the maturation of the germ cell the points where the interpretations diverge are, (1) an early stage when the double spireme develops, and (2) a later stage when the tetrad becomes formed. These two stages will be designated for the sake of convenience as the 'lepto-zygotene' and 'pachy-streptotene' stages, respectively.

The condition of the chromatin threads at the 'lepto-zygotene' stage was considered, not only by Meves ('96, '07, '11), Kingsbury ('98, '02), Duesburg ('08), Fick ('07, '08), and others, but also by telosynaptists, as representing a longitudinal splitting and as essentially the same as in the corresponding stage of prophase in homotypic mitosis. Parasynaptists claim that this is the stage when a parallel conjugation, two by two, of simple chromatin threads takes place.

As stated before, there is no evidence of conjugation, nor of splitting, in the case of Perla. The condition here might best be described as the development of a double spireme out of the chromatin reticulum of a resting nucleus, although the duality of the spireme may be best interpreted as a precocious splitting. In the first place, there are no definitely formed fine undivided leptotene threads in the nucleus before the double threads begin to appear. It is true that there are many "thick and often double threads terminating in two undivided diverging thin threads like the branches of a Y, which often separate at a wide angle and may be traced for a long distance," but these are hardly adequate to base the conclusion that parasynapsis is taking place, because the condition may just as well, or better, be attributed to the rearrangement of the reticulum into double spiremes. As a matter of fact, the thick threads may be seen diverging into more than two thin threads, as observations by Fick ('07) and also by Janssens and Dumex ('03) have shown. It must be concluded that, at least in the case of Perla, there is no evidence of synapsis at this stage. Also, since there are no clearly differentiated simple threads before the development of

the double threads, an actual longitudinal splitting of such threads cannot account for the production of the double spireme.

According to the view of parasynaptists, the 'pachystreptotene' is the stage when each spireme segment becomes open along the line of synapsis. Modern telosynaptists believe that each segment with two chromosomes conjugated end to end up to this stage bends at the synaptic point, and finally the constituent chromosomes come to lie side by side. The space enclosed by a ring at this stage should result from a longitudinal opening up of a spireme segment, if parasynaptists are correct in their interpretation, or, if we take the telosynaptists' view, this should be the consequence of the bending of the spireme segment, two arms of which coming in contact with each other to form a ring. The process taking place in *Perla* is in accordance with the second view, as it is described in the last section.

It must be noted, however, that the conclusion that chromosomes conjugate telosynaptically can be only indirectly supported in the light of later history of the haploid spireme segments. The actual process of end to end conjugation of chromosomes has not been observed, and telosynapsis, therefore, shall still remain as an hypothesis.

CONCLUDING REMARKS

I have come to agree with the view of the telosynaptists in the case of *Perla immarginata*, reaching the conclusions that:

1. Homologous chromosomes are connected to each other telosynaptically in the spireme.

2. That later they bend toward each other at the synaptic point and become reunited parasynaptically before the metaphase, thus forming rings and tetrads.

If there be no error in my observation, and should my interpretation prove to be correct, the feeling is irresistible that at least some of the recent parasynaptists are misinterpreting the relation of the so-called 'primary' and 'secondary' splits in the tetrads and the nature of the split in the early spireme. It seems also possible that some of the very convincing figures of early prophase stages, those of Wenrich ('17, p. 517, figs. 1 to 4), for instance, may be found to be partially incorrect.

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PLATES

All the figures were drawn with the camera lucida on the level of the table, and with Zeiss apochromatic 1.5-mm. oil-immersion objective and no. 8 compensating ocular.

PLATE 1

EXPLANATION OF FIGURES

Spermatogonial division

- 1 A metaphase plate, with ten chromosomes.
- 2 Two cells in metaphase (right and middle), and one in anaphase (left).
- 3 Early telophase.
- 4 Late telophase; the nucleus is beginning to resume the appearance of 'resting.'

First spermatocytic division

- 5 A 'resting' cell, with its chromatin substance in the form of a reticulum.
- 6 to 13 Cells in early prophase (lepto-zygotene stage). Rearrangement of the reticular chromatin substance into a double spireme is taking place. Figures 12 and 13 show the polarized condition of the forming double spireme.
- 14 and 15 'Contraction stage.' The duality of the spireme is already completely established.
- 16 to 18 Degenerating cells.



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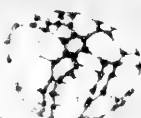
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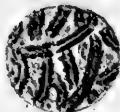
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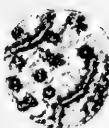
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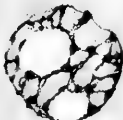
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PLATE 2

EXPLANATION OF FIGURES

First spermatocytic division—*Continued*

19 to 23 The formation of a double spireme completed (zygotene stage). There is a peculiar chromatin body in the nucleus at this stage, as is represented by the nucleolus-like structure in figures 19, 20, and 23, or by a modified portion of the spireme in figure 22. The exact nature of these structures has not been worked out, although their genetic relation with accessory chromosomes seems very probable.

24 to 26 Breaking up of the spireme into segments (pachytene stage). The segments are in reduced number.

27, 31, and 32 Halves of each spireme segment bending toward each other, the original split of the spireme is clearly recognizable (pachystreptotene stage).

28, 29, and 30 Transformation of spireme segments into tetrads; the narrower lateral split in the tetrads undoubtedly corresponding to the original split of the spireme.

33 A cell entering the metaphase.

34 Metaphase spindles. The split of the early spireme is still visible on the chromosomes.



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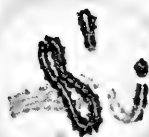
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PLATE 3

EXPLANATION OF FIGURES

First spermatocytic division—*Continued*

- 35 to 37 Metaphase plates.
- 38 to 40 Metaphase spindles. These may show that the split line of the spireme has nothing to do with that of the division of chromosomes. Figures 39 and 40 illustrate the separation of the X- and Y-chromosomes.
- 41 Early anaphase of the division.
- 42 Later anaphase.

Second spermatocytic division

- 43 The stage immediately following the anaphase of the first division. Bipartite chromosomes are already visible as such.
- 44 A cell just before the metaphase (left) and another in metaphase.
- 45 A metaphase spindle, showing equational division of chromosomes.
- 46 Metaphase plates with five chromosomes, including the Y.
- 47 Ditto, including the X.
- 48 Anaphase of the division.
- 49 Later anaphase.
- 50 to 51 Spermatids.



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Resumen por el autor, Sidney Isaac Kornhauser,
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Los caracteres sexuales del membrácido *Thelia bimaculata* (Fabr.)

I. Cambios externos inducidos por *Aphelopus theliae* (Gahan).

El drínido poliembrionario *Aphelopus theliae*, deposita un huevo en la ninfa del membrácido *Thelia bimaculata*. Un solo huevo produce de cincuenta a setenta y cinco larvas que viven en el abdomen del animal parasitado. Los individuos parasitados de *Thelia* se transforman a menudo en adultos, pero se modifican por la presencia de las larvas de *Aphelopus*. Los machos se parecen a las hembras por el color, tamaño, forma y costumbres. La transformación se extiende hasta los pequeños detalles del exoesqueleto. Los órganos genitales externos en ambos sexos se reducen considerablemente en tamaño y pierden sus caracteres específicos. Los órganos genitales de la ninfa durante la última muda son inhibidos a menudo en su desarrollo hasta que llegan a parecerse a los de la muda anterior. Los parásitos inhiben también el crecimiento de las gonadas en ambos sexos. Los testículos sufren una degeneración parcial o completa, pero en ningún caso los espermatocitos producen células parecidas a los ovocitos. Los machos parasitados almacenan grasa en sus abdómenes hipertrofiados y son menos activos que los normales. Las modificaciones mas marcadas tienen lugar en los caracteres específicos de los machos; después siguen las de los detalles de los órganos genitales externos de ambos sexos; los caracteres menos modificados son los de las células germinales no maduras. Los primeros procesos ontogénicos no se modifican bajo la acción de los parásitos en vías de crecimiento pero los caracteres que aparecen más tarde en la ontogenia se modifican profundamente. El cambio de metabolismo inducido por los parásitos es mas anormal en el macho que en la hembra y produce un efecto marcado sobre aquel ser altamente especializado inhibiendo la acción de los genes filogenéticos recientes que le comunican sus caracteres sexuales específicos.

THE SEXUAL CHARACTERISTICS OF THE MEMBRACID, *THELIA BIMACULATA* (FABR.)

I. EXTERNAL CHANGES INDUCED BY *APHELOPUS THELIAE* (GAHAN)

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FIFTY-FOUR TEXT FIGURES

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1. INTRODUCTION

In the insects and especially in the Homoptera, the supposed physical mechanism for sex determination is well known. Most of the forms show a single x-chromosome in the male diploid group and two x-chromosomes in the female diploid group. A wide difference of opinion exists as to the part these chromosomes play in sex differentiation. One investigator will speak of them as sex determiners, another will allow that they are merely associated with sex, a morphological expression of the underlying sex and comparable only to other structural sexual differences. The present paper is an attempt to throw some light on sex determination in insects.

Let us first define the characteristics which distinguish maleness from femaleness. The male germ gland produces sperm cells, the female produces eggs, and that certainly is the primary difference. But in addition there are sexual differences in the soma and these are striking in the group of insects under consideration. These somatic differences are generally designated as secondary sexual characteristics. They may be divided into two categories: those immediately concerned with the transfer of the spermatozoa or the laying of the egg may be called genital secondary characteristics, and those differences in color, ornamentation, and general form of body may be called extragenital secondary sexual characteristics or, if one choose, tertiary sexual differences. One of the problems, stimulated by remarkable results in the vertebrates, has been to determine in the insects, whether or not the development of the somatic sexual differences is dependent upon or independent of the primary sex gland.

Attempts to alter these secondary and tertiary characteristics of insects by experimental castration or the transplantation of gonads have not proved successful: the soma seems to be fixed, either male or female, and not dependent for its development upon the gonad nor upon some hormone developed by the gonad. We have, therefore, no means, except through hybridization, under our direct control of determining whether or not the secondary sexual characteristics of the opposite sex may be present in a latent form in either male or female insect. Certainly, latent characteristics cannot be brought out, as they may be in birds and mammals, by removal or transplantation of the gonads. Accordingly, it is of interest to observe the results of an experiment in nature in which the seemingly fixed and striking sexual characteristics of the male insect have been lost and those of the female have appeared in their place; this change being brought about by the action of internal hymenopteron parasites.

In the summer of 1911, while collecting *Thelia* at Cold Spring Harbor for a cytological study, the writer was struck with the fact that more gray females were to be seen on the locust trees than handsome brown and yellow males, and this in midseason,

a time when the males should be at their maximum. Examination of the gray individuals showed that some were altered males, each of which, when dissected, revealed the presence of thirty-five to fifty larvae in its abdomen. A great deal more material was observed and collected in the summers of 1914-17, and it is upon this material that the present paper is based.

We are first concerned with the external changes which the parasites bring about in their host, and to this I shall attempt to confine the present study; secondly, with the internal changes produced, to which I shall at times refer and upon which it is hoped more data can soon be procured; and, thirdly, to the nature of the parasites themselves, about which a number of facts are now known and are briefly given in part 5.

2. SYNOPSIS OF PREVIOUS PAPERS ON THE SEX OF ARTHROPODS

Experimental observation on the relation between germ gland and soma in insects goes back to Oudemans ('98), who castrated both male and female caterpillars of gypsy-moths. Although the objection may be raised that the majority of the larvae were castrated only on one side, yet neither these nor the more important, completely castrated individuals showed in their adult form any deviations from the normal structure or instincts. Morphological and psychological sexual characteristics were not in the least altered by the absence of the gonads. Oudemans reviewed in his paper many of the striking cases of gynandromorphism then known and used these as additional evidence to prove that in insects the soma is independent of the germ plasm in its development. Cramptons' paper ('99), while not dealing with the castration of Lepidoptera, has an interesting bearing on the question of sex in the insects. He grafted the pupae of a number of our common moths in pairs arranged in tandem and side by side. The members of a pair were often opposite sexes of the same species, others were opposite sexes of different species. In some cases the ovaries of the female component grew into the male portion of the graft, and yet in none of the cases in which the imaginal stage was successfully reached were the colors of the components in the least altered from the normal.

Likewise, small ingrafted portions of integument retained their original color and the transfusion of haemolymph was also without effect on the color of the adult. In the bisexual grafts, a free flow of haemolymph from one component to the other was possible, and the conclusion to be drawn is that the presence of the soma, haemolymph, and gonads of one sex will not inhibit the development of the characteristics of the other component belonging to the opposite sex.

Kellogg ('04), by the use of a heated needle, castrated the larvae of silk worms. The animals which survived the operation and became adults did not show any alterations from the normal in their sexual characteristics. Meisenheimer ('09) published the results of extended researches upon the castration and transplantation of gonads in moths. He used a platinum needle heated by electricity to burn out the small gonads, and this he could do successfully on minute caterpillars after their first molt. Castration was often followed by the implantation of gonads of the opposite sex. Testes developed normally in the female soma, likewise ovaries developed in the abdomen of males, but the eggs were always smaller and fewer in number than normal. Castration alone or castration followed by ingrafting gonads of the opposite sex had no effect on the secondary sexual characteristics; structure and breeding instincts remained unaltered. A series of experiments in which the anlage of a wing was destroyed in each larva is significant. The anlage regenerated in individuals of three types, those with normal gonads, those without any gonads, and those provided with gonads of the opposite sex, and yet in all cases where an adult wing was formed, it bore the original coloration and pattern of the sex operated upon. In his general consideration of the soma and germ plasm of insects, Meisenheimer reviewed important papers on arthropod gynandromorphs and assembled in his publication many of the best illustrations of external and internal conditions found in these anomalous individuals.

Regen ('09 a, '09 b) castrated nymphal crickets before their final molt or before their penultimate molt. He allowed the individuals which bore identification marks to mature in their natural

habitats. In his second paper he is very positive that the absence of the gonads has no effect upon the adult. Castrated males chirped as loud as ordinary males; their mating instincts were normal; they produced spermatophores, although no sperm was present to fill them, and the stridulating apparatus remained unchanged. Castrated females also had their normal structure and habits, and even bored in the ground with their ovipositors, although no eggs were present in their bodies. Regen's work is the only one on paurometabolic insects.

Turning again to the Lepidoptera, Kopeć has an interesting series of papers ('11, '13 a, '13 b), mainly on *Lymantria dispar* L. and *Gastropacha quercifolia* L. He developed a remarkable technique in the removal of the gonads, using a sickle-shaped hook and for the smallest larvae a hook of silver wire. For the transplantation of the gonads, he used sterile pipettes. Castration and implantation of gonads of the opposite sex were performed on larvae of first, second, or third larval stage. Often he would repeat the ingrafting of gonads so that the abdomen and thorax of the operated individual would contain many instead of two gonads. Testes grafted into castrated females often grew to be larger than normal testes and in their histological structure were normal. Ovaries which developed in the bodies of castrated males were always much smaller than normal ovaries. There were seldom more than one-fourth or one-fifth the normal number of ova developed and these were small. Sections showed that their yolk granules were fewer and smaller than in normal eggs. In *Gastropacha quercifolia*, the ova developed in the male soma were yellow instead of green. The small size of the implanted ovaries, Kopeć maintains, is due entirely to lack of space for development in the male abdomen. The haemolymph or extract of triturated gonads, when injected into castrated individuals of the opposite sex, produced no effect upon the adult structures. In all his experiments (castration, castration followed by implantation, and transfusion) the results are in perfect agreement with those of his predecessors and strengthen the idea that in insects the development of the secondary sexual characteristics is in no way dependent on

the gonads nor on hormones from the gonads. In more recent experiments Kopeć ('13 a) removed the imaginal disc of the left antenna from larvae which were castrated and into which gonads of the opposite sex were grafted. The regeneration of the antenna supported his former conclusions as to the independence of the soma, in that most of the antennae were normal in form and also showed their characteristic coloration—light in the male and dark in the female. A few females developed light antennae, and Kammerer ('13) attacked the conclusions of Kopeć, using these individuals for his argument. Kopeć ('13 b), by a series of check experiments, successfully answered Kammerer's objections by showing that in control females, not castrated, the regenerated antenna at times were light instead of dark. This condition is, therefore, not due to the absence of the normal gonad, but doubtlessly is caused by a trophic effect brought about by the operation.

Another interesting and instructive line of work throwing light on the physiology of the sexes in the insects is that of Steche ('12) and of Geyer ('13), who experimented with the haemolymph of various insects, mainly Lepidoptera. Steche noted that the haemolymph of male larvae of *Lymantria dispar* was yellow, that of the females green. The yellow pigment was shown to be xanthophyll, the green pigment a metachlorophyll formed from the leaves eaten by the caterpillars. Besides this color difference, there are protein differences between the sexes, shown by bringing together in a watch-glass the haemolymph of male and female larvae. At contact there was thrown down a 'veil-like' precipitate. The stiffening of larvae into which foreign haemolymph is injected may be explained by the formation of this precipitate. The sexual differences in color and protein content of the blood were shown to be independent of the gonads, for they remained unaltered in castrated individuals and castrated individuals with implanted gonads of the opposite sex. Steche attributes these sexual differences to the somatic cells which produce the haemolymph; thus, cells of the female digestive tract allow the chlorophyll to pass through quite unchanged, whereas only the xanthophyll passes through the cells of the

male digestive tract. Thus the somatic tissues of insects are clearly sexually differentiated, and this differentiation is independent of the gonads. It is, therefore, superfluous, according to the author, to speak of primary and secondary sexual differences in the insects, for all differences are primary—those of the soma as well as those of the germ plasm. Geyer ('13) extended the observations of Steche to other Lepidoptera and also to other orders of insects, including rapacious forms in which no color differences existed in the haemolymph. He also carried out extensive castrations, transplantation of gonads, and transfusion experiments. His results are in perfect agreement with those of Steche. Even where no color difference exists, a precipitate is formed in bringing together the haemolymph of opposite sexes. This precipitate is often quite as dense as that formed by mixing the haemolymph of different species or genera. He also showed that, where color differences existed in the blood, it was not due to an enzyme in the male destructive to the color found in the female haemolymph, for in no case could the meta-chlorophyll be bleached by the addition of male haemolymph. These observations of Steche and Geyer would seem to indicate that a male soma would be unable to furnish the coloring matter or the complete protein requirements for ova transplanted into such a soma. An interesting question might also be raised in regard to the characteristics of the haemolymph in perfect lateral gynandromorphs of Lepidoptera, such as described by Toyoma ('05), and in which doubtless half of the cells of the digestive tube are male and the other half female.

Turning now to Nature's own experiments on sex, gynandromorphs, we have a definite line of evidence in the insects and Crustacea supporting the independence of somatic development from gonad influence. A complete analysis of the individuals, including the description of the gonads as well as external characteristics, is to be sought for in a study of the biology of sex. Such a description is given by Wenke ('06), whose article adequately describes and illustrates the conditions found in his *Argynnis* gynandromorphs. A perfect lateral gynandromorph contained a single well-developed ovary which did not in the

least interfere with any of the secondary sexual characteristics of the male half of the animal. As will be pointed out later, such examples as these have an important bearing upon the reasons given for changes seen in parasitized crustaceans and upon theories of sex based on general metabolic differences. Many cases similar to that of Wenke, and others presenting different internal conditions, namely, the presence of testes or the presence of both testes and ovaries, have been described and many of the most important cases up to 1909 reviewed by Meisenheimer ('09). Recently, Duncan ('15) has reported on several interesting gynandromorphs in *Drosophila*. Male and female soma together may be associated with the presence of either testes alone or ovaries alone. In the *Lepidoptera*, Cockayne ('15) has presented every possible association of somatic sexual mixture with gonads of one or both sexes. In the lower Crustacea, Bremer ('14) describes two individuals of *Diaptomus*: the first had male somatic characteristics associated with the presence of an ovary; the second a female abdomen which contained a testis. Such anomalous forms of arthropods have interested not only entomologists and students of sex, but also geneticists and cytologists. The causes of gynandromorphism are generally looked upon as having a chromosomal basis. Boveri ('15) believed that, in the lateral gynandromorphic Eugster bees, the spermatozoon united with one of the two daughter nuclei formed by the parthenogenetic division of the female pronucleus, and that this zygote gave rise to the female half of the individual, whereas the unfertilized daughter nucleus produced the male half. His contentions are upheld by showing that the external characteristics of the female half are hybrid; those of the male half, maternal. Morgan ('16), basing his contentions on Toyama's ('05) silkworm gynandromorphs produced by crosses of different races, believes the male half in these cases to be formed by a supernumerary spermatozoon developing parthenogenetically in the egg cytoplasm, as the male half is paternal in its external characteristics. That parthenogenesis in moths can give rise to males has been shown by Goldschmidt ('17 a). Probably the 2x condition is obtained in the male half by a doubling

of the chromosomes introduced by the spermatozoon. Sex intergrades, which are generally mosaics of the soma of both sexes, often containing abnormal gonads of hermaphroditic character, have been studied by Goldschmidt ('16, '17 b) in the moth *Lymantria*, and by Banta ('16) in the crustacean *Simocephalus*. Although these forms, as in the case of gynandromorphs, support the idea that the somatic cells are physiologically independent of the gonads in development, still the explanation of these mosaics on a cytological basis must await further investigation of their chromosomal make-up. Banta believes the environment plays an important rôle, whereas Goldschmidt (17 b), although believing in a chromosomal explanation, is skeptical of obtaining a visible demonstration of size differences of chromatic elements in the components of the mosaics of *Lepidoptera*.

Although the sexual characteristics seem to be fixed, there are nevertheless three lines of evidence which indicate that in every normal individual the determinants for the opposite sex are present in a hidden condition. First, the males may be produced by parthenogenesis, as is the case in the rotifers, in many Entomostraca, and in numerous insects, especially homopterans and hymenopterans, also occasionally in *Lepidoptera* and artificially induced in frogs (Loeb, '16, Gatenby, '17). The mechanism of parthenogenetic male production has been most fully solved in the aphids (von Baehr, '09) and in the phylloxerans (Morgan, '09). That the egg before maturation is equivalent to the genetic constitution of the cells of the female which produced it is agreed upon by all biologists. In the production of a male from such an unfertilized egg, something must be eliminated to allow the hidden male characters to appear. Thus Morgan and von Baehr have shown that there is a differential maturation and that every small (male) egg throws off a whole x-chromosome or a group of x-chromosomes in the polar body. We can hardly escape from the belief that the presence or the absence of a particular x-chromosome determines whether the male or the female characteristics shall develop in the mature egg. The size of the egg, however, regulates the maturation, so it seems; since the small eggs always extrude one x-chromosome or one group of

x-chromosomes, whereas in the large egg all the chromosomes divide equationally and a female results. This idea does not assume that the x-chromosomes actually contains the determinants for the sexual characteristics, primary or secondary, but that they merely influence the development of these characteristics, which are in all probability borne by the autosomes.

The second line of evidence is gained from breeding experiments. Harrison and Doncaster ('14) have shown in *Ithysia zonaria*, in which the females alone are wingless, that the male of this moth (when crossed with the female of *Lycia hirtaria* winged in both sexes) transmits to his daughters a characteristic of the *zonaria* females, namely, small flightless wings, much smaller than those of their parents. Foot and Strobell ('14, '15, '17 a, '17 b) have crossed several species of *Euschistus* in which they have studied the inheritance of two 'exclusively male characters.' They have shown that the length of the intromittent organ and a black spot on the male genital segment may be transmitted through the female as well as through the male. What weight the term 'exclusively male character' carries is hard to gather from the papers, since, according to the interpretation of the authors, it includes characteristics at one time equivalent to primary sexual characteristics and at another time equivalent to sex-linked characteristics. The intromittent organ we would call a genital secondary sexual characteristic, whereas the spot we would call an extragenital secondary sexual characteristic or even a tertiary sexual characteristic. Foot and Strobell's arguments against the chromosomal basis of heredity will be considered in the discussion. The facts of their breeding experiments show very nicely that, just as in birds and mammals, the female may transmit the male characteristics of her species in crosses. The determiners for these characters must, therefore, be present in her genetical make-up, although they were not expressed in her soma.

We now come to the third line of evidence: the effect of parasites on the sexual characteristics. There are three important papers dealing with the strepsipteran parasites of Hymenoptera. Perez ('86) describes and pictures the modifications in *Andraena*

brought about by *Stylops*. The head showed a reduction in size, the abdomen became more globose with the puncturing less strongly marked, and the villosity increased. The scopa, or pollen-carrying apparatus, of the hind tibia of the female was reduced in parasitized females. These individuals also lost the pollen-gathering instinct. In the males, on the contrary, the narrow hind tibia was increased by the presence of *Stylops*. The clypeus of the female lost its black color and gained the yellow color of the male, whereas the clypeus of parasitized males showed merely a reduction in the extent of the yellow pigment. The sting of the female was reduced greatly in size. This was also the case with the external genitalia of the male. Perez contends, therefore, that not only is there a loss of secondary sexual characteristics due to the parasites, but also in certain cases there is the assumption of characteristics of the opposite sex. A study of the gonads showed that in the male one testis might continue functional, whereas in the female only a minute rudimentary ovary remained.

Wheeler ('10) studied the effect of *Xenos* on *Polistes*. He also gives a most excellent review of work done on the castration of insects. The parasitized *Polistes* failed to give the interesting series of changes we might expect. They merely assumed a reddish tinge to the abdomen and face. Wheeler's work does not, of course, invalidate that of Perez; it merely fails to extend the known changes on bees to the wasps. Smith ('14) studied three species of *Andraena* infested with *Stylops*. A large part of his study is devoted to the development and habits of the parasite and the remainder to the internal and external changes wrought in the host. He reviews in detail the work of Perez and reproduces several of his figures. Of the parasites themselves, two facts are of especial interest. *Stylops* carries on its respiration with the external world through two tubercles on the head, which extend between two abdominal segments of the host, and therefore does not use the haemolymph of its host for respiratory changes. The sex of the parasite is not a factor in considering the changes brought about in *Andraena*. Smith does not describe modifications as extensive as those given by Perez, but in

two changes both authors agree: the reduction of the scopa of the female and the assumption of the male color for the clypeus of the female in *Andraena chrysosceles* and *Andraena labialis*. Stylopized males show no reduction of the testes and may have functional sperm; but in the female the ovary, which is normally a hundred times the size of the testis, is greatly reduced through lack of nourishment and produces only minute functionless eggs. Smith seizes upon this fact as the cause for the changes wrought in the female characteristics. He argues that, as in birds (Goodale, '16) the ovary inhibits the development of male characteristics, so also in *Andraena* the absence of the ovary allows the male characteristics to develop. This assumption will be considered later.

Most closely associated with the study undertaken in the present paper is the work of Giard ('89). He described the effect of the internal parasitic dryinid, *Aphelopus melaleucus*, and the parasitic dipteran, *Atelenevra spuria*, on the homopterans *Typhlocyba hippocastani* and *Typhlocyba douglasi*. In females of both species of *Typhlocyba* infested with *Aphelopus*, the ovipositor was much reduced. *Atelenevra* had much less effect on this organ. In *Typhlocyba hippocastani* the oedagus is a complicated forked organ, and this is greatly altered by the parasites, the forks being reduced from eight branches to six, four, or even three. In the males of both species there occurs on the ventral wall of the abdomen a pair of organs of unknown function, perhaps homologous with the sound-producing apparatus of male cicadas. Ordinarily, these extend from the first to the posterior extremity of the fourth somite. In parasitized males these enigmatical organs seldom reach beyond the middle of the first somite, being reduced to two small pockets. Matausch ('09, '11) described the effect of insect parasites on Membracids. In his first paper he believed that he was dealing with gynadromorphs, but later ('11) discovered that the abnormalities were caused by parasites.

Changes similar in character, but even more striking than those described in the insects, have been studied in crabs infected with rhizocephalans, parasitic barnacles. Giard ('86, '87 a, '87 b,

'88), Potts ('06), and Smith ('10) show conclusively that infected males develop the secondary sexual characteristics of the female. The abdomen assumes the general form characteristic of the female, even bearing biramous abdominal appendages. The large chela of the male is replaced by the slender claw of the female. Smith ('11, '13) and Robson ('11) have studied the effect of the parasite on the lipochromes, fats, and glycogen content of *Carcinus* and *Inachus*. In parasitized *Carcinus* males the yellow lipochrome characteristic of the female blood appeared. The fat content of the blood and liver increased, whereas the glycogen content decreased. These changes show that the metabolism of the parasitized animals had become female. Smith believed that the roots of the parasites made a demand upon the soma of the host similar to the demand for fat made by an ovary. The response to this demand, the assumption of female metabolism, carried with it the production of female secondary sexual characters in the morphology of the host. Altered metabolism brought about changes in the blood of the male which stimulated the development of the latent female characteristics. It is not the absence of the testes, but the presence of the parasite acting like an ovary which brings about the changes in the males.

Not only rhizocephalans, but also protozoa may alter sexual characteristics. Thus Smith ('05) described changes in the crab *Inachus* due to a gregarine. The abdomen and claw of the male were altered much as described above for the barnacles. Only those individuals in which sporozoites were liberated in the haemolymph showed modifications. No case exactly parallel to this is known in insects. Grassi and Sandias ('93) maintained that the presence of Protozoa in the intestinal caecum prevents the full development of both internal and external genitalia in termite workers. When the Protozoa are killed or removed by feeding saliva, the purged individuals become sexually mature substitute kings and queens. Wheeler ('10) was inclined to believe that the dimorphism in the males of *Forficula*, based on length of the forceps, might be due to the gregarines infesting their alimentary tracts. Brindley and Potts ('10) do not believe

in this assumption since they found no correlation between the length of the forceps and the number of gregarines in adult insects. It might here be interjected that, since the adult structures of insects cannot be altered, it would be necessary to know how many gregarines had been present in the alimentary tract previous to the final molt, at which time their presence might influence the imaginal structure.

3. MATERIAL AND METHODS

A brief description of the life history and habits of *Thelia* and its parasite *Aphelopus* will be given in parts 4 and 5. The principal collecting ground was situated about two and a half miles from the laboratory, and material was procured practically every second day and brought in alive. For further observations on living specimens, the insects were placed in cages on cut locust branches or put upon branches of locust trees growing near by, and enclosed in bags of cheese-cloth or mosquito netting. The branches in the cages must have the cut ends in water and the leaf surface reduced to remain fresh. They must be renewed every second or third day to keep the animals in good condition so that they will grow and molt.

For a study of the parasitized adults and normal individuals, first the pronotum with head and prothorax attached was removed, pinned, numbered, and shielded from the light to protect the colors, which fortunately keep well in dried specimens. The body was placed immediately in a dish of physiological salt solution or in Ringer-Locke solution, and dissected under a binocular microscope. The abdomens were cut open dorsally with a fine microscissors so as not to injure the genitalia. A careful search was then made for any remnants of gonads in parasitized individuals. The light from a Nernst glower directed upon the interior of the animal by a condensing flask added greatly in discovering any minute gonads which might be present. The gonads discovered were removed and fixed for sectioning: testes in Bouin's fluid, ovaries in Gilson's fluid. The body and parasites were separately preserved for further study, usually being put into 80 per cent alcohol. Body, pronotum, and

gonads of an individual were all given the same number and a careful record kept of date, fixation, characteristics of pronotum and genitalia, and size of parasites, together with any exceptional condition worthy of record. This was done for every specimen.

Nymphs of all stages were fixed whole in Petrunkevitch's fluid warmed to 50°C. The duration of fixation varied, with the size of the nymphs, from one hour for the smallest up to twenty-four hours for the largest.

In making preparations of the genitalia, the non-chitinous portions were removed by heating in a solution of caustic soda and then trimming the preparation with a fine scissors and scalpel under a binocular microscope. They were then dehydrated and mounted in balsam.

Sections were made through the bodies of adults and nymphs. In this work the celloidin-paraffin method (Kornhauser, '16) was invaluable. Sections were made 10μ in thickness, and it is possible to cut the hardest chitin without tearing the ribbons or nicking the knife. The gonads were cut 6μ in thickness, and stained in Heidenhain's haematoxylin and Congo red.

All the figures (with the exceptions of numbers 7, 8, 18, 32 to 35, 53, 54) are untouched photographs made with Spencer micro-teleplat objectives 8 mm., 24 mm., and 60 mm. In the 24-mm. and 60-mm. objectives, iris diaphragms were inserted. The non-photographic figures are drawings made with the aid of a camera lucida.

4. BRIEF ACCOUNT OF THE LIFE HISTORY AND HABITS OF THELIA

Thelia bimaculata is the largest and one of the commonest membracids of northeastern United States. It feeds exclusively, as far as is known, on the sap of the common locust (*Robinia pseudo-acacia* L.). It is found on the trunk or larger branches of this tree. Adults occur from July to October. Association with other organisms is nicely seen in this docile, domesticated homopteran which is constantly attended by ants and is imposed upon by internal and external parasites. Hymenoptera live within its body, mites attach themselves to the exterior, and

predacious dipterans often pounce down and carry the *Thelia* away without much trouble.

In my principal collecting fields, *Formica truncicola* Nyl. subsp. *obscuriventris* and *Cremastogaster lineolata* Say were the chief ants associated with *Thelia*. When tapped by the antennae of the ants, the *Thelia* nymph or adult exudes from the anal tube a drop of clear fluid which is taken by the ant with great alacrity. Toward the middle of June, the ants build collars about the bases of the locust trees, and inside these collars in the cracks of the bark are to be found hundreds of *Thelia* nymphs of third to fifth instar, quietly feeding and undisturbed by the numerous ants in attendance. In this moist situation, protected from many of their enemies, the nymphs thrive. *Formica* builds the protecting collar of leaves, twigs, and bits of wood; *Cremastogaster* builds of sand grains cemented together. When one breaks the collar, many ants swarm out and attack the intruder, *Formica* biting one's fingers ferociously, while others grab the *Thelias* and drag them into underground passages. These pugnacious ants seem to have complete mastery of the *Thelia* nymphs.

After completing its growth in the fifth instar, the *Thelia* emerges from the collar of leaves or sand, climbs higher on the trunk or branches of the tree, and molts into an adult. Mature males are first to appear, generally being found early in July. The females and parasitized adults of both sexes mature a week or two later. In July and early August, the adults sit motionless on the bark and are not found in groups. One can often catch them between the forefinger and thumb. The males, however, are more active than the females and hop or fly at less provocation. Both sexes are more active on hot, sunny days. Toward the end of August and in September, the individuals gather into groups on the branches or the trunk of the tree, and there is evidence of courtship, for very often one sees a single female surrounded by several males. In September, individuals mating can occasionally be found. The male and female face in opposite directions and the tip of the abdomen of the male is placed beneath the ninth abdominal segment of the female.

Thelia lays its eggs in late September and in October. At this time the males are already becoming less numerous. The female lays her eggs in the bark of the small branches of *Robinia*. I have never seen eggs being deposited at the bases of trees as described by Funkhouser ('15), who has written a very good account of the life history of *Thelia* and has described the five instars. With her sword-like ovipositor the female makes a slit through the bark, longitudinal to the branch and tangential to the underlying wood part. The total length of the egg is 2.4 mm. The chorion forms a tube 0.4 mm. beyond the contained ovum, which is, therefore, but 2 mm. in length. This chorionic tube projects from the slit in the bark and probably aids in the respiratory changes of the developing embryo. From three to six eggs are deposited in a single slit and one *Thelia* will lay between thirty and forty eggs at a time, judging from dissections of adults previous to laying. The eggs remain in the bark over winter. In early June they hatch, and the small shiny brown nymphs begin to feed out on the small branches of the tree. They occur in cracks in the bark at the bases of thorns, or at the edges of healed wounds where the bark is thin and succulent. First, second, and third instars occur on the branches, constantly attended by ants. Soon, however, the ants begin to build the collars at the bases of the trees and third to fifth instars are found in abundance only inside these collars. As described above, they emerge as full-grown nymphs in July, crawl higher on the tree and molt into adults.

5. *APHELOPUS THELIAE* (GAHAN), A POLYEMBRYONIC PARASITIC DRYINID; ITS LIFE HISTORY AND HABITS

The life history and habits of *Aphelopus theliae* were gradually worked out by the author until at the present time we have a fairly complete story. The difficulty in getting the series of events in the life cycle complete was due to the fact that until the past summer (1917) the stay at Cold Spring Harbor had not begun early enough in June to obtain adults and to see the laying of the *Aphelopus* egg in the nymphs of *Thelia*.

Aphelopus theliae belongs to the Dryinidae, characteristically parasitic on homopterans, but never before known to be polyembryonic. This species was named and described by Mr. A. B. Gahan ('18) from material reared and sent by the author.

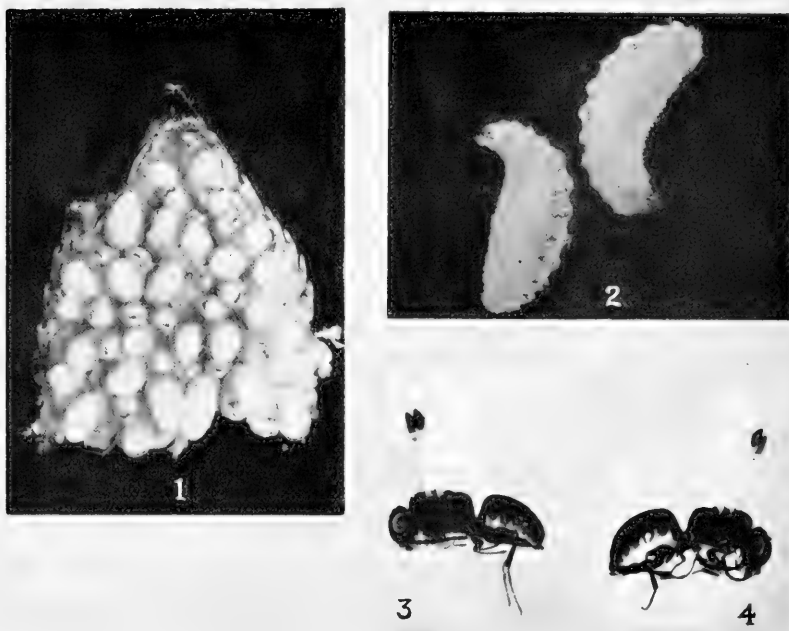


Fig. 1 Megagnathie larvae in abdomen of *Thelia*. Terga, digestive tube, and parasites dorsal to digestive tube have been removed. $\times 9$.

Fig. 2 Eruciform larvae as they emerge from their host. $\times 8.7$.

Fig. 3 Male *Aphelopus theliae*. Taken from pupa case before complete transformation accomplished. $\times 10.1$.

Fig. 4 Female *Aphelopus theliae*. Taken from pupa case before its ova had reached maturity. $\times 10.1$.

Imagoes are shown in figures 3 and 4. They are jet black. The female is 2.2 mm. in length, the male slightly shorter. The specimen shown in figure 4 was taken from its pupa case before complete maturity was reached. This accounts for the

space between the abdominal terga and sterna; the fat distending the abdomen shows through laterally at this stage, but not in sexually mature individuals.

In early June, when the young *Thelia* nymphs of first to third instar are abundant on the smaller branches, the female *Aphelopus* hurries nervously up and down the branches of the locust tree hunting its prey. Finding a nymph, she taps it with her antennae, much as does an ant, and quiets it; she then climbs upon the dorsum of the nymph, feels its head with her large powerful mandibles, to test, I believe, whether or not the nymph is soon to molt. If the parasite decides to deposit an egg, she grasps the caudal part of the nymph's abdomen between her mandibles, and, holding firmly with her legs to the abdomen of the host, tries to thrust her sword-like ovipositor cephalad through the intersegmental membranes of any two abdominal terga. The nymph struggles as the ovipositor pierces, and from the anal tube exudes a drop of liquid. This, the *Aphelopus* grasps in her mandibles and is gone in a second. If the nymph is a small first, second, or third instar, the whole act of ovipositing may take but a few seconds; but, when a fourth or fifth instar is attacked, a long struggle often follows and it may take several minutes before the small energetic parasite accomplishes her task. Having examined the nymph and decided to oviposit, the *Aphelopus* gets hold of a tibia with its mandibles and legs and hangs on, no matter how hard the *Thelia* kicks or even if both go rolling over and over. The parasite then tries to force its ovipositor through the soft membranes between the trochanter and femur or between the coxa and thoracic sternum. After ovipositing, the *Aphelopus* generally mounts the abdomen of the nymph to secure a drop of excrement. After a struggle with a large nymph, the parasite remains on the bark or on a leaf, cleans her wings, legs, antennae, and mandibles, and especially rubs her ovipositor vigorously with her hind legs. The whole process of oviposition was watched many times in the laboratory under a binocular microscope by putting a female *Aphelopus* into a test-tube containing a locust twig upon which several *Thelia* nymphs were feeding. The parasite would hunt

over the twig, and finding the nymph would oviposit just as in nature. This procedure was continued by renewing the nymphs until the *Aphelopus* was exhausted. She might oviposit again the next day, laying in all from twenty-five to fifty eggs, generally one in each nymph. It was found best to work the parasites as hard as possible the day they were captured, for they were most active then and would live only a few days in the laboratory. One lived five days, but that was unusual. The only successful way to capture the adult female *Aphelopus* was to place the mouth of a small vial over the individual as soon as she was discovered running over the bark, and thus try to corner her so that she would run up into the vial. One must not hesitate a second nor await a more favorable opportunity, for, should an ant come along, the parasite for which one may have been hunting several hours would hop or fly in terror. *Aphelopus* has a keen fear of ants, especially of *Formica*. This was tested in the laboratory by putting an ant and an *Aphelopus* into a tube. The ant immediately took the offensive, showing that an enemy of *Thelia* is not to be tolerated.

When one dissects, under a high-power binocular microscope, a nymph which has just been stung, one will find a thin-shelled oval egg, 145μ in length and 60μ in diameter, and also several spheres covered with a chitinous shell and filled with yolk-like material. These spheres are developed in the female *Aphelopus* from single cells in a sack-like pocket ventral to and leading into the posterior portion of the oviduct, just below the opening of the spermatheca. The function of the spheres, which vary from 25μ to 35μ in diameter, has not yet been determined. The egg¹ absorbs fluid from its host and the thin shell swells. Within, total cleavage takes place and the sphere of cells formed soon develops into a polygerm mass. This mass becomes oval, and then angular and irregular in outline, as it starts to form branching chains of embryos. These chains are composed of spheres connected and covered by a placental envelope several cells in

¹ A detailed study of the cleavage of the *Aphelopus* egg and the formation of the polygerm is contemplated, suitable material having been secured from laboratory-stung specimens during the past summer.

thickness. This envelope constricts between the embryos and the chains are broken up into separate individuals, each of which develops into a larva surrounded by its nutritive envelope. The details of this growth and differentiation will be left to a subsequent paper.

If the *Thelia* nymph is stung during the first or second instar, the abdomen of the fourth instar will be filled with forty to sixty larvae bent in a half-circle, ventrad and measuring 0.75 mm. from the cephalic to the caudal end. Each embryo is enclosed in a semitransparent envelope of cells which doubtlessly serves as an organ of nutrition and respiration. The mouth parts are not chitinized at this stage. In the fifth instar or the adult *Thelia* the larvae reach their maximum development as internal parasites (fig. 1, p. 548). They grow tremendously and acquire large, hard, brown, chitinous mouth parts, well-defined stigmata, and a circumcephalic plate (Keilin and Thompson, '15) which in its dorsal region is brownish and thrown into many transverse folds. On account of the prominent mouth parts, we may designate the larvae of this stage as megagnathic larvae. Surrounded by fat, they are packed close to one another, about thirty-five being in contact with the abdominal sterna of the host, fifteen or more occupying the region lateral and dorsal to the digestive tube, while occasionally a few are present in the thorax. Reaching their maximum size, they distend the abdominal segments of the host to such an extent that the intersegmental membranes are clearly visible between the abdominal sclerites. This must certainly interfere with the respiratory movements of the host.

The megagnathic larvae have a well-defined alimentary tract which ends blindly caudad. The tract is filled with shining crystals which appear white in reflected light. These crystals are kept in constant motion by peristaltic waves which may be viewed nicely in living larvae in Ringer's solution. A deep constriction of the digestive tube runs caudad, then reverses and runs cephalad. The crystals in the tube are very insoluble, persisting in specimens kept for several years in 80 per cent alcohol and resisting in section-making all the ordinary reagents.

They are probably some of the katabolic substances formed in the parasites' development which are stored in an insoluble condition rather than being thrown into the haemolymph of the host, as the host might be unable to rid itself of these waste products.

The final molt of the parasitic larvae and their escape from the host is the next act. This generally occurs during the fifth nymphal instar of *Thelia*, but if oviposition of the parasite occurs late in the ontogeny of the host, the *Aphelopus* larvae reach maturity in the adult *Thelia* and escape often with some difficulty from the imagos. The fact that the parasites may be present in adults and are found there in various stages of development makes this paper possible. In *Lepidoptera*, for instance, the larvae or pupae are always destroyed by their polyembryonic parasites, and so we cannot tell how the sexual characteristics of the imago might have been affected. The *Thelia* nymph or adult from which the larvae are soon to emerge leaves its comrades, climbs to a solitary twig or leaf, and fastens itself firmly with its tarsi. Small elevations running in rows across the abdominal sterna appear. A little later each elevation becomes a hole from which the caudal end of a green or yellowish eruciform larva emerges. Before escaping the larvae devour the entire contents of the host, leaving only an empty shell clinging to the leaf or twig. By wriggling motions, the larvae work their way out of the host and also out of the integument of the megagnathic stage. The integument is broken in the dorsal region behind the circumcephalic plate and the eruciform larva leaves its exuvia at the hole through which it emerges from the *Thelia*. The chitinous jaws of the exuviae plainly mark the holes made in the sternites of the host (figs. 43 and 47). The eruciform larvae (fig. 2) are entirely different in form from the megagnathic larvae, having small jaws and a very bristly integument marked into distinct segments by rings of spines. They are yellow or light green, depending upon the amount and color of the pigment which was present in the body of the host. On the average, thirty-five larvae free themselves simultaneously, the abdomen of the host bending until the ventral surface is par-

allel to the ground and allowing them to drop to the earth. These are followed by the remaining individuals which did not have a ventral position in the host next to the abdominal sterna.

The eruciform larvae crawl at a good rate, hunting any small opening in the ground. In the laboratory it was found advisable to make small holes in the soft earth in jars into which the larvae dropped. Finding these holes, they burrow half an inch or an inch through the soil and spin a little straw-colored cocoon, 2.36 mm. in length. The cocoon is almost oval, but a trifle constricted about the middle where a thickened portion forms a little white transverse band. Often the larvae spin their cases against stones or the side of the jar furnished the laboratory cultures. One could follow the color changes of the enclosed larva or pupa, for the cocoon is quite transparent along its surface of adherence.

By the end of September, the larva has transformed itself into a white-bodied pupa with red or chocolate-colored eyes. The abdomen is filled with fat and the intestine is distended with crystals and dark unorganized waste material. Evidently the winter is passed in this state, the transformation to the jet-black adult taking place in the spring. In the laboratory cultures kept in a warm greenhouse, development continued. Thus, by December the chitinous portions of the *Aphelopus* were completely formed, but the abdominal sclerites were still distended with the enclosed fat. In the males sexual maturity was already reached, spermatogenesis having been completed; but the females contained only minute ova. In April the adults hatched in the laboratory; the females then contained fifty to seventy full-grown eggs. From these specimens reared in 1916, before the adult had been seen in nature, the species was named and described by Mr. Gahan.

Female *Aphelopus* hunting *Thelia* nymphs, taken in June, 1917, were dissected and revealed the fact that in some the spermatheca was filled with sperm and in others it was empty. Probably fecundated females lay eggs which develop into females, whereas virgin females lay eggs which develop partheno-

genetically into males. The offspring of a single egg, as tested in laboratory cultures, were all of the same sex. However, as noted by Patterson in *Paracopidosomopsis* ('17), both sexes may emerge from a single host, and this would not be impossible in the case of *Aphelopus*, for at times a female might oviposit in a nymph already containing an *Aphelopus* ovum. This was demonstrated in the laboratory and both eggs were recovered by dissection. Twice in parasitized *Thelia* secured in nature *Aphelopus* larvae of two distinct stages of development have been found in single individuals. In these cases only those coming from the first ovum would become adults, for the host would be killed at their emergence; but, should two ova be laid within a short time in the body of a *Thelia*, it would be possible that some offspring of both ova would reach maturity at the same time and produce a mixed brood.

6. CHANGES INDUCED BY APHELOPUS IN THE COLOR AND FORM OF THE INTEGUMENT AND APPENDAGES OF THELIA

A. *The thorax*

In *Thelia*, as in most membracids, the pronotum forms a conspicuous part of the organism. It is continued forward and upward as a horn projecting beyond the head, and it also extends caudad as the posterior process covering the dorsum of the entire thorax and abdomen. The pronotum constitutes one of the most conspicuous differentiating characteristics of the extragenital type between the sexes. In the male (fig. 5) it has a uniform, chocolate-brown color with a conspicuous orange-yellow vitta on each side, extending caudad from the humeral angle usually about half-way to the tip of the posterior process. The length and form of the pronotal horn as well as the angle it makes with the rest of the body are subject to the greatest variation, as is shown in figure 7. Sometimes it is long, curved, slender, and erect, (7, *d*, 7, *h*); again, short, straight, and blunt (7, *e*, 7, *l*). The form of the vitta is also variable, the yellow color showing many degrees of extension toward the tip of the posterior process. Figure 7 is a series arranged from *a* to *l* to

show this variation. Yet, in spite of this lack of uniformity, the vitta is always present in normal males and the rest of the pronotum is a uniform brown. The light areas in figure 5 out-

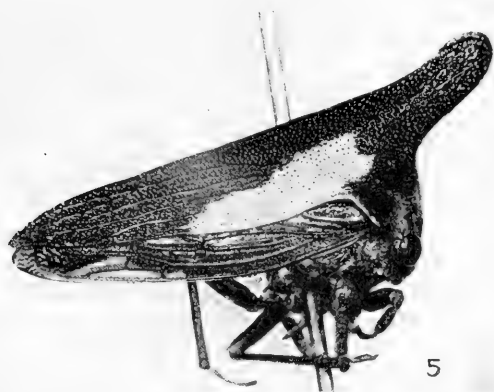


Fig. 5 Normal male *Thelia bimaculata*. $\times 6$.

Fig. 6 Normal female *Thelia bimaculata*. $\times 6$.

side the vitta are merely reflections of light. The whole pronotum is covered with coarse punctures which are nicely seen on the vitta of figure 5. If, now, we examine a vertical section of a portion of the pronotum, including part of the vitta and

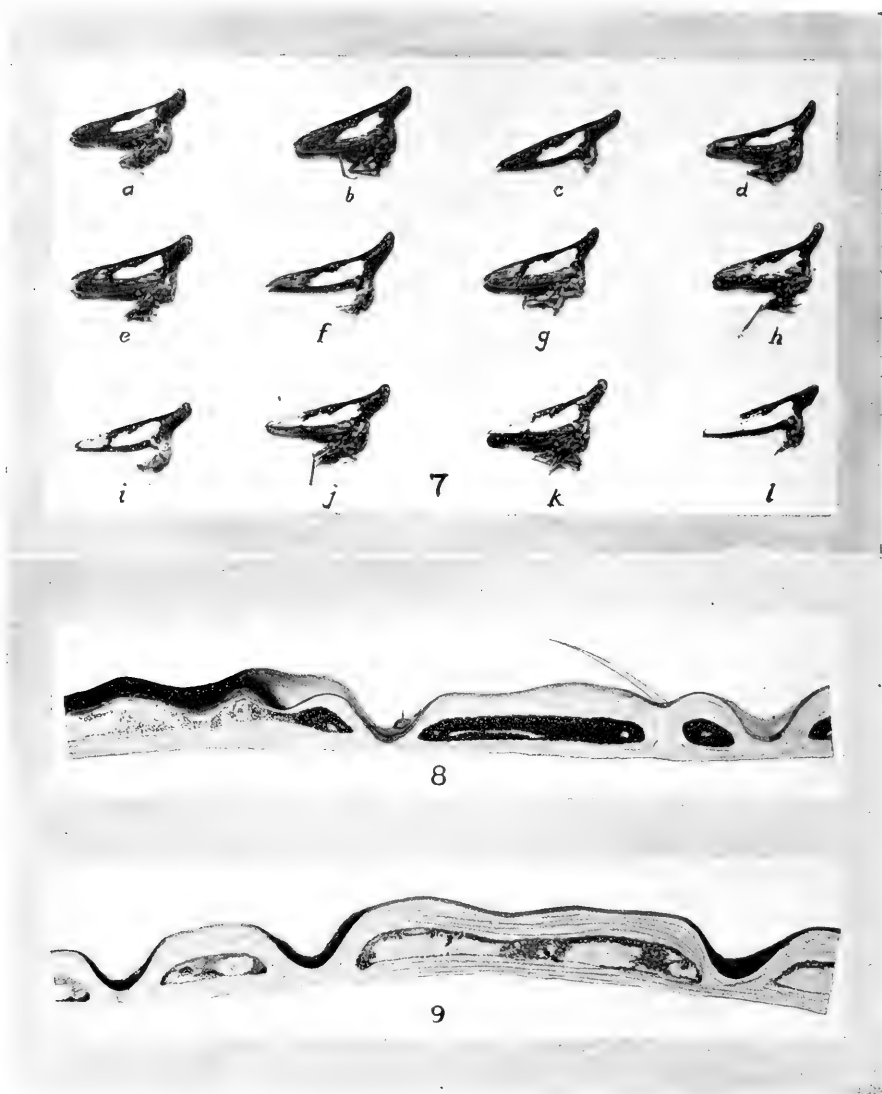


Fig. 7 Normal males and pronota of normal males, showing variation in extent of vitta and form of pronotal horn. Extension of yellow pigment in vitta arranged in series from *a* to *l*. $\times 1.5$.

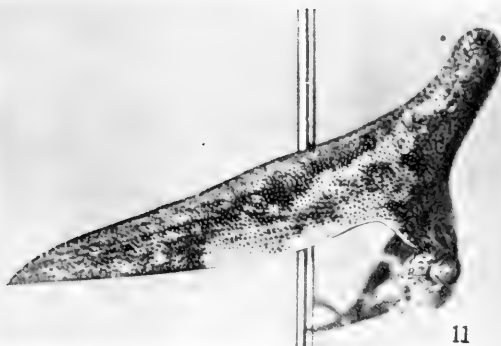
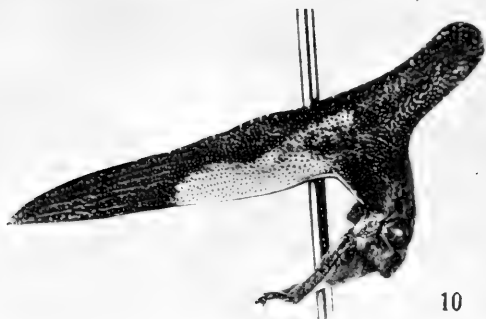
Fig. 8 Vertical section of cuticle of pronotum of male, passing through part of vitta. $\times 144$.

Fig. 9 Vertical section of cuticle of pronotum of female, passing through part of vitta. $\times 144$.

the bordering brown area (fig. 8), we shall see that the brown pigment is contained in the upper layers of the chitin in an amorphous form. This is present over the whole surface outside the vitta in the punctures and intervening areas. This brown pigment is melanin, resisting strong alkalis and acids. In the hypodermal spaces below the brown chitin red granules are embedded in a clear matrix which surrounds the tracheae extending through the pronotum. In the vitta the chitin is transparent, no melanin being present, and the hypodermal spaces are filled with an orange-yellow granular pigment which shows through the chitin and gives that area its striking color. This hypodermal pigment is easily destroyed by acids and alkalis and gradually loses its color in alcohol. In figure 8 the hypodermal pigment, which may vary from a light yellow to a deep orange-yellow, is shown in the right half of the section. The punctures in the vitta are very transparent, there being no hypodermal pigment present and just a trace of melanin in the most superficial layers of the chitin, which produces a yellowish tinge.

In the female (fig. 6) the pronotum has a gray tone, harmonizing nicely with the bark upon which the animal rests. The vitta is not conspicuous, being but a trifle lighter than the rest of the body. A vertical section (fig. 9) shows us how the gray coloration is brought about. The melanin of the entire pronotum is restricted to the punctures and the edges of these punctures, whereas the hypodermal spaces are partly filled with a yellow-green granular pigment. Some red granules may also be found in the hypodermal matrix immediately surrounding the puncture, and must in some way be associated with the presence of melanin in the cuticula above. The combination of the brown punctures and greenish-yellow areas produces a gray tone in the pronotum.

One of the most striking effects of *Aphelopus* is causing the pronotum of the male to assume the pigmentation of the female pronotum. Many steps in the transformation have been seen in parasitized adults and several are shown in figures 10 to 12. Some individuals are but slightly affected (fig. 10), others have



Figs. 10, 11, and 12 Pronota of parasitized males, showing loss of male characteristics and assumption of female pigmentation and size. Figure 10, slight loss of uniformity of melanin and loss of hypodermal yellow. Figure 11, greater loss of uniform melanin and further encroachment of melanin on vitta. Figure 12, complete loss of male characteristics and complete assumption of those of female. $\times 6$.

perfect female coloration (fig. 12), while many show merely a medium condition (fig. 11). In slightly modified males the yellow hypodermal pigment of the vitta becomes fainter and less abundant, and melanic spots appear in the cuticula of the vitta (fig. 10). In individuals showing greater assumption of female coloration the melanin loses its uniform distribution outside the vitta, becomes restricted more and more to the punctures, and encroaches still farther upon the vitta. Yellow-green hypodermal pigment forms and shows through the cuticula no longer impregnated with melanin (fig. 11). Finally, in completely altered males, the punctures alone are brown (fig. 12) and the hypodermal pigment is exactly like that of the normal female in color and distribution. These changes are summarized in figures 13 and 14. Figure 13 represents ten vittae seen in reflected light, and figure 14, the same in transmitted light. Yellow pigment appears light in figure 13, and dark in figure 14, since it absorbs the actinic rays of the transmitted light. Melanin is dark in both figures. The clear punctures of the male vitta are light in transmitted light (fig. 14, *a*). Vitta *a* in both figures is that of a normal male; vitta *j*, that of a normal female. Vittae *b* to *h*, inclusive, are parasitized males and illustrate the gradual loss of yellow accompanied by the coming in of melanin in the punctures. In *g* and *h* complete assumption of female coloration has taken place. Vitta *i* is that of a parasitized female. It shows no tendency toward the assumption of male pigmentation. At most parasitized females show smaller punctures with restricted melanic pigment and a thinner and weaker cuticula. This is doubtlessly due to an interference with the normal nutrition of the hypodermal cells which produce the chitin, as a similar condition may often be noted in parasitized males. In these cases there may be an actual scarcity of the necessary materials for chitin and pigment production, caused by the presence of the parasites.

It must be clearly borne in mind that no modification in the integument can be effected by the parasites after the host has become an adult. The degree of change is, therefore, dependent upon the activity of the parasites previous to the final molt of

the Thelia. It is during the fifth nymphal instar that preparation for the most striking feature of the metamorphosis of the homopteran occurs (compare fig. 15 with figs. 5 and 6). The

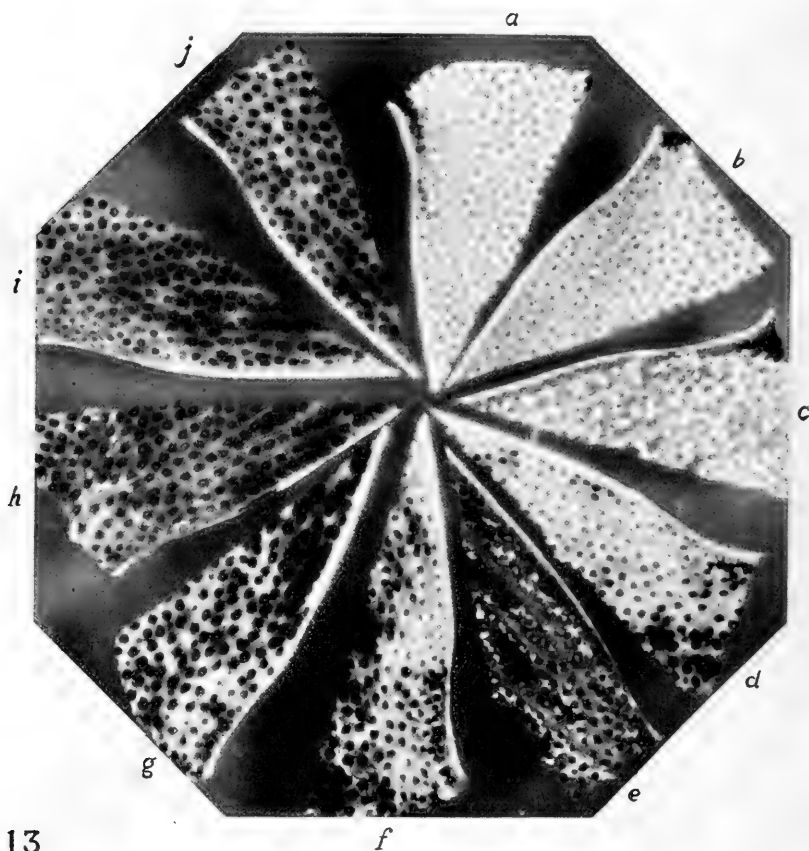


Fig. 13 Vittae mounted in balsam, seen in reflected light. *a*, normal male; *j*, normal female; *b* to *h*, inclusive, parasitized males showing various degrees of loss of yellow hypodermal pigment (light in color in photographs) and increase of melanin in punctures; *g* and *h*, complete change; *i*, parasitized female. $\times 16.6$.

sexual differences of the pronota as well as many other remarkable changes in the integument appear first at the final molt. If, for example, the parasites in a male fifth instar are large

megagnathic larvae while the preparation for this transformation is going on, female coloration and many other changes to be described are brought about. If, on the other hand, the Aphe-

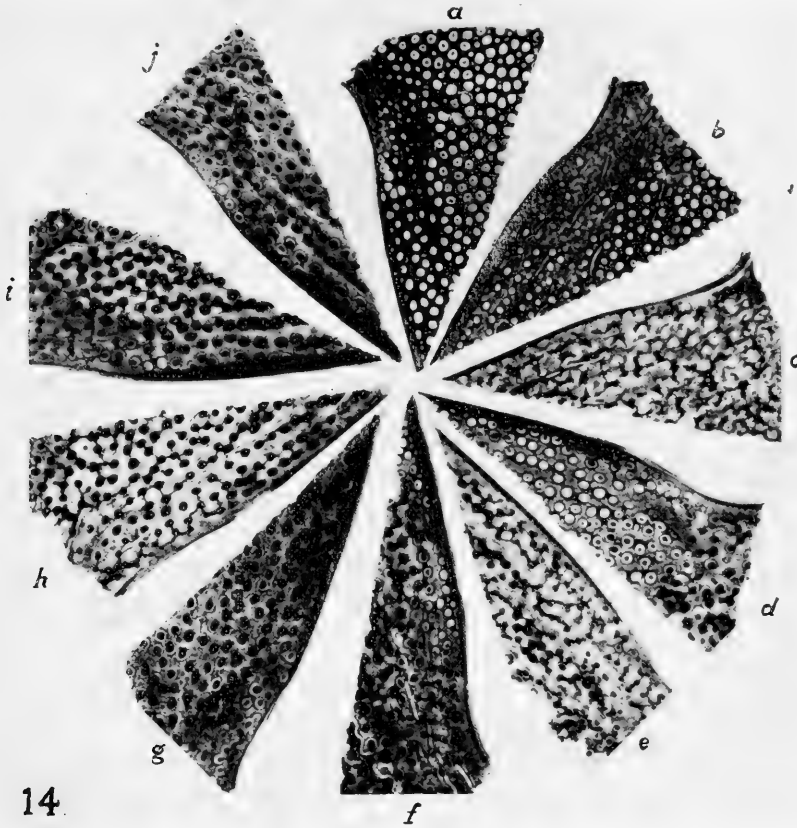


Fig. 14 Same as figure 13, seen in transmitted light. Chitin without melanic pigment (punctures in normal male), light; yellow pigment acting as absorber of actinic rays appears dark between punctures when present; melanin also appears dark. $\times 16.6$.

lopus has oviposited in a nymph during its fourth or fifth instar, the parasites will be small and have less effect upon the adult structures.

The changes in the coloration of the male pronota above described cannot be referred to retardation of development for the integument of the fifth instar (figs. 15 to 18) is entirely different in structure and coloration from that of the adult. The cuticula covering the entire dorsum of the nymph has long spines projecting from its surface (figs. 17 and 18). These spines are

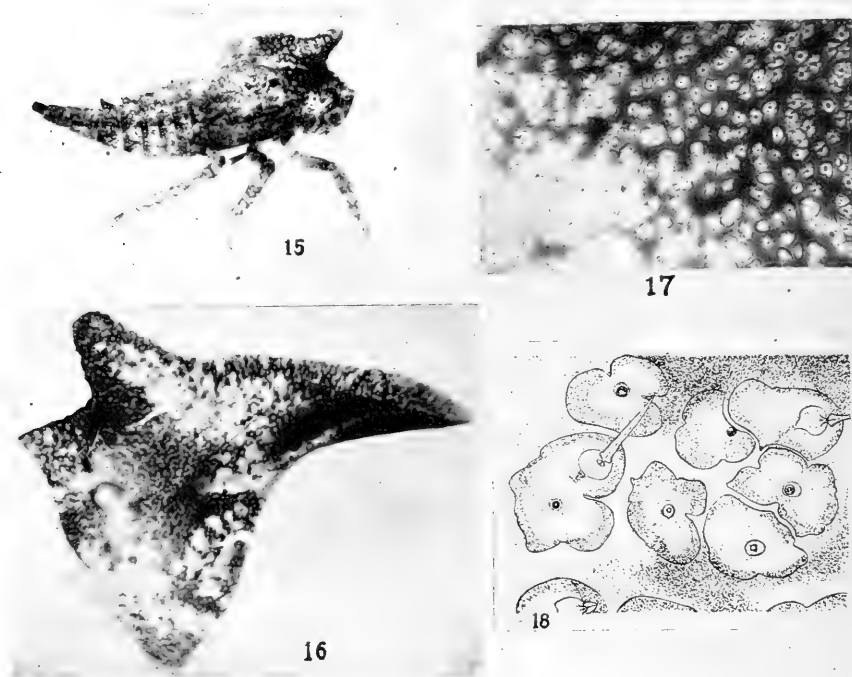


Fig. 15. Lateral view of fifth nymphal instar, showing mottled pigmentation of body and appendages due to large areas devoid of melanin. $\times 5.3$.

Fig. 16. Cuticula of pronotum of fifth nymphal instar. Each small spot on the dark background represents the base of a long jointed hair. The large light spots which cause the mottling are plainly shown. $\times 14.7$.

Fig. 17. Small area of cuticula seen in figure 16, under greater magnification, showing details of long jointed hairs in dark and light areas. $\times 66.6$.

Fig. 18. Cuticula from sixth abdominal tergum of nymph in fifth instar, showing jointed hairs, their bases, and distribution of meanic pigment. $\times 600$.

jointed near their bases. The melanin is very irregularly distributed, always being absent about the bases of the spines and also in larger irregular areas, which produces a mottled appearance over the whole nymph (figs. 15 and 16). Parasitized adults possess none of these juvenile characteristics, but males affected by *Aphelopus* assume female coloration through the loss of male characteristics and the addition of those of the adult female integument.

Female *Thelia* are larger than males. Thus the pronota of 111 normal females, measured with a micrometer caliper from the tip of the horn to the end of the posterior process, averaged

TABLE 1

	NUMBER OF INDIVID- UALS	AVERAGE LENGTH OF PRONOTUM	AVERAGE WIDTH OF PRONOTUM
		<i>mm.</i>	<i>mm.</i>
Normal males.....	114	11.55	4.73
Parasitized males showing medium or complete change to female coloration.....	98	12.24	5.03
Parasitized males with male coloration still pre- dominating.....	29	11.68	4.85
Total parasitized males above.....	127	12.11	4.98
Normal females.....	111	13.39	5.41
Parasitized females with decidedly reduced ovi- positor.....	100	13.11	5.33

13.39 mm., and the average width across the humeral angles was 5.41 mm. (table 1). Corresponding measurements on 114 normal males gave 11.55 mm. as average length and 4.73 mm. as the width. This shows that the pronota of normal females are about 15 per cent longer and broader than those of normal males. If, now, we examine parasitized males with a well-defined change in color (medium to complete), we see that they are both longer and wider than normal males, the increase being about 6 per cent. That this is due to the action of the parasites is shown by the fact that in males parasitized late in their ontogeny (those with male coloration still predominating) we find but a slight increase in size. Only when large parasites are

present during the fifth nymphal instar is there a decided increase in length of the pronotum. The effect cannot be produced by mechanical means, because the parasites are present in the abdomen, whereas the pronotum is attached to the prothorax alone and receives its material for growth through the haemolymph coming into it through the prothorax. The increase in the size of the pronota in parasitized males is of still greater interest when we see that in the parasitized females there is no increase, but rather a decrease of about 2 per cent in both length and breadth. We are, therefore, led to the conclusion that the 6 per cent increase in size in the males infected with parasites is a partial assumption of a female characteristic.

TABLE 2

	NUMBER OF INDIVID- UALS	AVERAGE LENGTH OF FOREWING
		<i>mm.</i>
Normal males.....	25	8.13
Parasitized males showing medium or complete change to female coloration.....	25	9.09
Parasitized males with male coloration still predominating...	15	8.39
Normal females.....	25	9.78
Parasitized females with decidedly reduced ovipositors.....	25	9.81

Corresponding changes in size and color hold also for many other parts of parasitized male *Thelia*. The length of the fore wing was next examined. Measurements were made by removing the wings, laying them on a scale divided into tenths of millimeters and taking the reading under magnification. The length from the posterior tip to the anterior end of the tegula was used as the basis of comparison. The averages are given in table 2 and a typical example shown in figure 19.

The fore wing of normal females is on the average 20 per cent longer than that of normal males. Parasitized males with changed color show an increase of 12 per cent in length over normal males. Parasitized males with but slight change in color have wings only 3 per cent longer than normal males. In the case of the females, the parasites cause almost no change, those measured showing an increase of three-tenths of 1 per cent

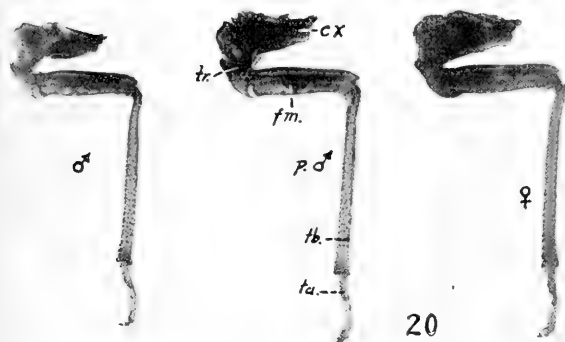
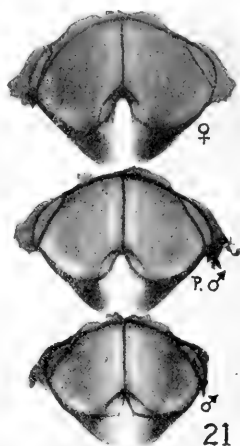
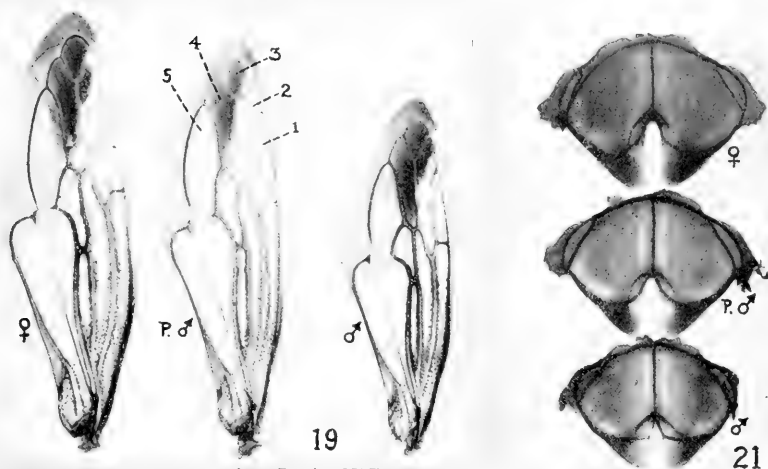


Fig. 19 Typical fore wings, showing increased length in parasitized male (p. ♂). Compare with normal male and normal female wings. From left to right, normal female, parasitized male (p. ♂), and normal male. 1, 2, 3, 4, 5, apical areoles. $\times 6$.

Fig. 20 Typical hind legs, showing size difference between male and female and increase in parasitized male. From left to right, normal male, parasitized male (p. ♂), and normal female. *cx.*, coxa; *tr.*, trochanter; *fm.*, femur; *tb.*, tibia; *ta.*, tarsus. $\times 7.1$.

Fig. 21 Typical acrotergites, showing approach toward female size in parasitized male. From top to bottom, normal female, parasitized male (p. ♂), normal male. $\times 7.5$.

over normal females. The wing length in parasitized males behaves just as did the size of the pronotum and the degree of change is correlated with the change in the color of the pronotum. Pronotum color is the best index one can find for practically all the changes in the male.

A qualitative change in the wing is also seen in parasitized males (fig. 19). Whereas in normal males melanic pigment extends diagonally through the second apical areole to the base of the fourth apical areole, in the female it forms only a spot in the second and is restricted to the distal two-thirds of the fourth areole. Parasitized males show a distribution of pigment like that of normal females.

Turning to the other thoracic appendages, the legs, we find that those of normal females are longer and stouter in every segment than those of the male. The size relation in the third pair is shown in figure 20. Similar to what was observed in the pronota and wings, parasitized males exhibit an increase in the size of the legs. This increase is especially noticeable if we compare the length of the tibia and tarsus taken together in each of the classes shown in figure 20.

Not only do the thoracic appendages increase in size in parasitized males, but the thorax itself becomes larger. This is best measured by comparing the acrotergites of normal males, normal females, and parasitized individuals. This plate, which extends from the metanotum ventrad between the mesothorax and metathorax and serves for the attachment of locomotor muscles, gives us a good idea of the cross-section of the thorax and the relative surface provided for the thoracic musculature. A comparison was made of thirty acrotergites removed entire, ten from each of the three classes represented in figure 21. Not only do parasitized males show a noticeable increase in the size of the acrotergite, but even the form of the aperture through which the digestive tube passes and the contours of the thickened ribs of the chitinous plate become female in character. The increased acrotergite indicates, I believe, that the muscles which move the enlarged appendages have become larger than the muscles of normal males. Although dipterous parasites are

known to cause the degeneration of wing muscles in certain Acridiidae (Künckel d'Herculais, '94), making volitation impossible, parasitized *Thelia* can both fly and jump quite as well as ordinary females. To move the enlarged wings and transport the increased bulk of the body certainly larger muscles are necessary.

B. The head

If we examine the sexual distinctions in the heads of *Thelia* we find differences of color, pattern, and size. The hypodermal pigment is similar to that of the pronotum in the respective sexes, being orange-yellow on the vertices and clypeus of the male and greenish-yellow in the female. There is also a sexual difference in the distribution of the melanic pigment of the face (fig. 22.), which is not only darker brown in the male, but also more abundant and less scattered than in the female. On the vertex about each ocellus the male has a well-defined spot, and along the medium suture between the vertices there is a distinct line of brown pigment. From this vertical line there are two diverging arms bordering the upper edge of the clypeus and forming an inverted Y. The male clypeus has two distinct bands extending ventrad from the arms of this inverted Y. Along its lower angle and the borders of the genae it is deeply pigmented. In the female, the melanic pattern is less distinct, being present in smaller and more irregular patches. Especially is this noticeable on the lower border of the clypeus which is a mottled light brown in the female, deep solid brown in the male. Parasitized males not only lose the orange-yellow hypodermal pigment which is replaced by greenish-yellow pigment, but in fully altered specimens also exhibit the melanic pattern characteristic of the female head. These changes cannot be ascribed to the retention of juvenal characteristics, for the color, pattern, and structure of the integument of the nymphal head (fig. 22, *ny.*) differ greatly from those of the adult and resemble the nymphal integument of the thorax and abdomen as described on page 563.

A comparison of head widths, measured at the level of the compound eyes, reveals the fact that parasitized males show an

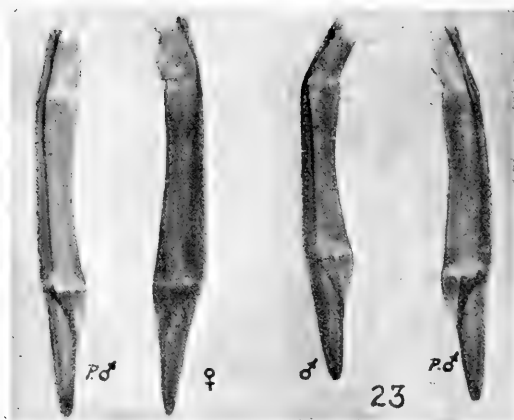
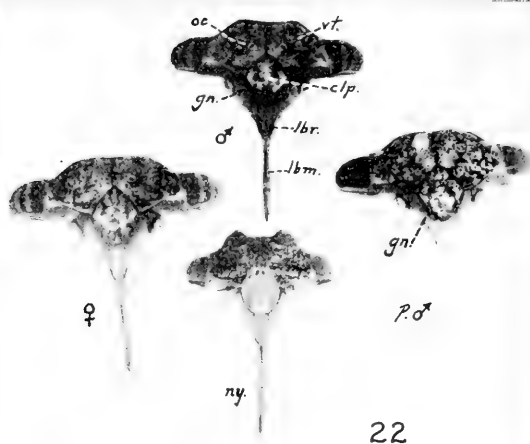


Fig. 22 Typical heads, showing male and female characteristics and effect of parasites on male head. Nymphal head included for comparison. Above in middle, normal male; below, nymph (*ny.*); left, normal female; right, parasitized male (*p. ♂*). *oc.*, ocellus; *vt.*, vertex; *clp.*, clypeus; *gn.*, gena; *lbr.*, labrum; *lbm.*, labium. $\times 7.5$.

Fig. 23 Labia, to show increased length in parasitized males. From left to right, parasitized male (*p. ♂*), normal female, normal male, parasitized male (*p. ♂*). $\times 21.7$.

increase in width approaching that characteristic for the female. This same relation of size increase is seen to exist when we compare lengths of labia of normal males, normal females, and parasitized males (fig. 23). As was the case in the pronota and wings, parasitized females show no marked changes in either size or pigmentation of the head.

C. Extragenital abdominal characteristics

The effects of the parasites are also very marked upon the abdomens of Thelia. The changes occurring in the extragenital secondary sexual characteristics or tertiary sexual characteristics will be described first.

The abdomens of the two sexes present very different appearances and, directly or indirectly, many of these differences are associated with reproduction. The female abdomen must be capable of holding, on the average, thirty-five ova, 2.4 mm. in length—a bulk much greater than that formed in the male abdomen by the testes, seminal vesicles, and accessory glands. In the male the reproductive apparatus is mature when the nymph molts to an adult, and is contained in the abdomen without distention; but in the female the ova are very minute at the beginning of imaginal existence and continue to grow, filling the abdomen and extending the chitinized abdominal sclerites to such an extent that the intersegmental membranes show as lighter bands between these pigmented plates. Thus the female abdomen is much larger than that of the male, and its cuticula is far more pliable than that of the male. This pliability, a necessity in accommodating the growing ova, is accompanied by a pigmentation of the abdominal sclerites, lighter than that of the male abdomen (figs. 24 and 28). Strength, firmness, and rigidity of chitinous parts in insects is always accompanied by a heavy melanic pigmentation, as in mandibles, ovipositing apparatus, and muscle attachments; whereas the absence of melanin leaves the chitin much weaker and more pliable.

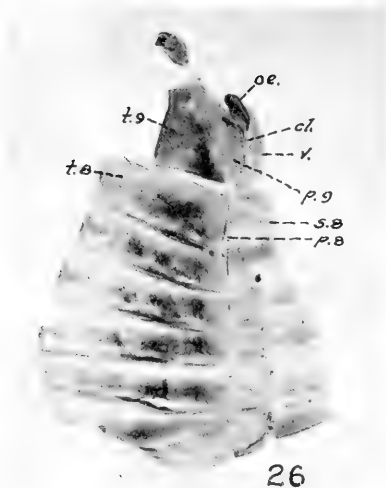
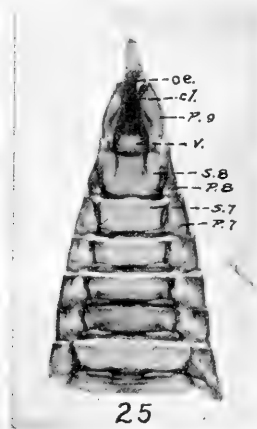


Fig. 24 Abdomen of normal male, lateral view of left half, compressed under cover-glass. *oe.*, oedagus; *cl.*, clasper or style; *v.*, ventral valves; *s.*, sternum; *p.*, pleuron; *t.*, tergum. 7, 8, 9, refer to abdominal somites to which sclerite or appendage belongs. $\times 9$.

Fig. 25 Abdomen of normal male, ventral half, not compressed under cover-glass. Abbreviations as in figure 24. $\times 9$.

Fig. 26 Abdomen of parasitized male, lateral view of left half, compressed under cover-glass. Abbreviations as in figure 24. $\times 9$.

Fig. 27 Abdomen of parasitized male, ventral half, not compressed under cover-glass. Abbreviations as in figure 24. $\times 9$.

Each typical abdominal segment consists ventrad of a sternum and two pleura (figs. 25 and 29, *s*, *p*) and dorsad and laterad of a tergum, bent into an arch which is somewhat more pointed at its apex in the female than in the male. The sterna of the female are much broader than those of the male. With the pleura which bear the spiracles, the sterna form a flat ventral surface which, at its union with the terga, forms a sharp ventrolateral angle. Thus the female abdomen is almost triangular in cross-section. In the male the sterna are bent slightly dorsad and the pleura are also bent upward, making them lateral rather than entirely ventral (fig. 25). No sharp angle is formed at the union of the male pleura with the terga. The abdomen is rather subovoid in cross-section. The sterna and particularly the terga of the male abdominal somites are more deeply pigmented than those of the female.

When we examine the abdomens of parasitized males (fig. 27) we see that the sterna increase greatly in width and that the abdomen in cross-section becomes similar to that of the female. The pleura become flattened plates entirely ventral in position and form with the terga a sharp ventrolateral angle. All the abdominal sclerites show a loss of pigmentation and a corresponding decrease in strength or rigidity. The terga (fig. 26) have even less melanin than those of most normal females, and the pigment remaining is restricted chiefly to the regions of muscle attachments.

Two of the changes effected by the parasites on males, namely, increase in size of the abdomen and decrease in firmness, strength, and pigmentation of the sclerites, are to be found even in individuals still showing predominatingly male characteristics in the thorax and head (figs. 36 to 39). Broad translucent sterna through which greenish fat and some red pigment show are an infallible clew to the presence of parasites. These changes are requisite to the development of *Aphelopus*. The larvae must have sufficient space in which to develop, and when full grown present a bulk quite as great as the ova of a mature female *Thelia*. The narrow abdomen of the male would be insufficient for the development of the polyembryonic brood which, before

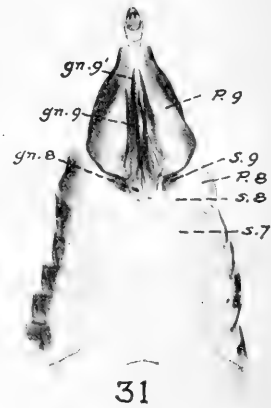
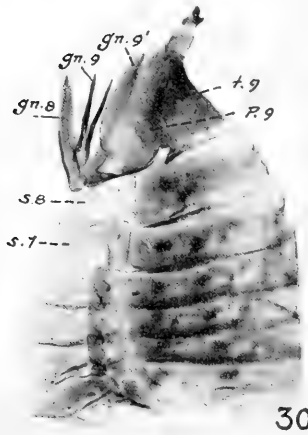
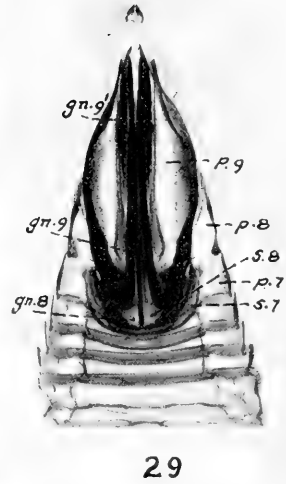
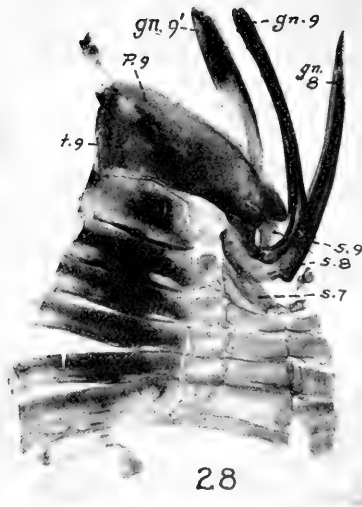


Fig. 28 Abdomen of normal female, lateral view of left half, compressed under cover-glass. *gn. 8*, gonapophysis of eighth segment forming left half of sheath surrounding ovipositor; *gn. 9*, ovipositor composed of anterior gonapophyses of ninth segment; *gn. 9'*, posterior gonapophysis of ninth segment which partly covers *gn. 8* and *gn. 9* when in natural position, as in figure 29; *s.*, sternum; *p.*, pleuron; *t.*, tergum; 7, 8, 9, refer to abdominal somites to which sclerites or appendages belong. $\times 9$.

Fig. 29 Abdomen of normal female, ventral half, not compressed under cover-glass. Abbreviations as in figure 28. $\times 9$.

Fig. 30 Abdomen of parasitized female, lateral view of right half, compressed under cover-glass. Abbreviations as in figure 28. $\times 9$.

Fig. 31 Abdomen of parasitized female, ventral half not compressed under cover-glass. Abbreviations as in figure 28. $\times 9$.

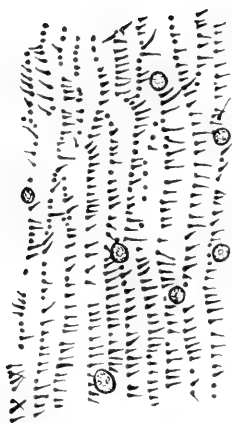
emerging, distends even the enlarged abdomen to its full capacity. Moreover, the larvae would be unable to bore through normally chitinized sterna, so these plates are thin and translucent in all parasitized individuals.

The abdomens of parasitized females (figs. 30 and 31) remain in cross-section and size similar to those of normal females. A loss of pigment is seen in all the sclerites and the sterna remain thin and delicate as in parasitized males to permit the escape of the larvae.

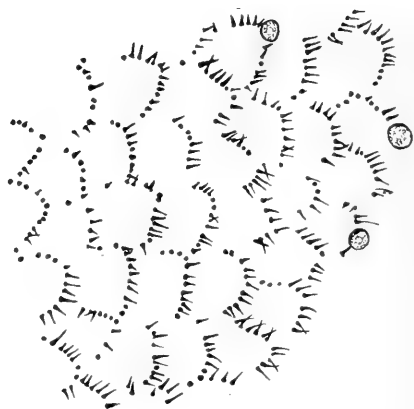
Turning now to a consideration of the details of the abdominal integument, we note two distinct sexual differences. The abdominal sclerites of the second to eighth segments, inclusive, possess long, scattered, chitinous hairs directed caudad, and in addition to these there are minute hairs forming very distinct patterns (figs. 32 to 35). These minute hairs are very differently arranged in the two sexes. Figures 32 to 35 are camera-lucida drawings representing a corresponding region of the sixth abdominal tergum of typical individuals. Figure 32 represents the arrangement found in normal males, fairly straight and compact rows running laterad and ventrad. The pattern on the female terga is strikingly different (fig. 34). The spines here form a network, made up roughly of rows of half-circles arranged alternately in such a way that the ends of the half-circles of one row touch the apexes of those in the row more anterior.

In parasitized males (fig. 33) there is a complete loss of the characteristic arrangement of these spines and almost a complete assumption of the female pattern. The only difference to be noted is that the ends of the arcs are not so well formed, usually lacking a few spines to complete the articulation with the row in front. This change of pattern is one of the clearest qualitative sexual changes in the abdomen of parasitized males and cannot be ascribed to anything except the assumption of a female characteristic. Parasitized females are similar to normal females in the above-described characteristic (fig. 35).

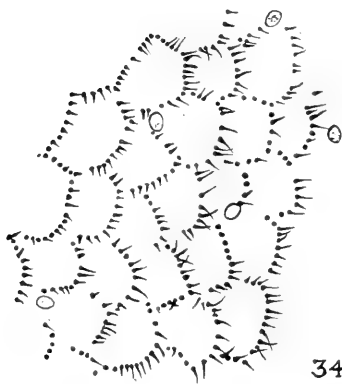
Short spines or hairs are also present on the sterna. In the male these are arranged in rows running in straight lines across the sterna from pleuron to pleuron. Camera-lucida drawings



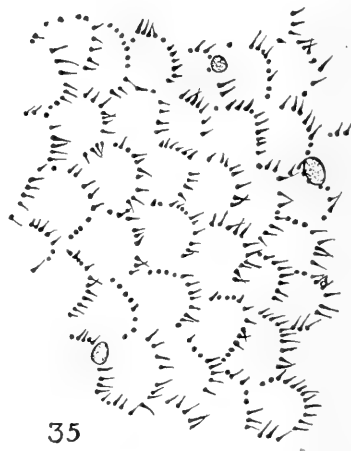
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33



34



35

Figs. 32 to 35 Surface view of integument of sixth abdominal tergum, showing typical arrangement of minute hairs in each class represented. All drawings from a corresponding dorsolateral area of the sixth abdominal segment. The dotted circles with light centers represent the long hairs which are scattered irregularly over the integument. Figure 32, normal male; figure 33, parasitized male; figure 34, normal female; figure 35, parasitized female. Corresponding area of nymphal integument shown in drawing in figure 18. $\times 480$.

show these rows to be on the average 7.5μ apart. In the females these rows arch cephalad on the sterna and are 9.3μ apart on the average. In parasitized males, instead of forming straight lines, they are curved as in the female and are 9.5μ apart.

These changes in the arrangements of the minute spines of male abdomens may not be ascribed to the retention of juvenal characteristics, for the integument of the fifth nymphal instar of both sexes is quite different from that of the adult. The terga of the nymph are covered with large jointed hairs (fig. 18, p. 562) and lack entirely the minute hairs arranged in patterns in adult terga. Also the nymphal sterna are clothed with peculiar triangular spines quite unlike those of the adult and not arranged in definite rows.

The sclerites of the eighth and ninth abdominal segments and the sterna of the seventh segment are quite dissimilar in the two sexes, being modified in each sex to accommodate the gonapophyses. Thus the sterna of the seventh and eighth abdominal somites are rectangular in the male (figs. 24 and 25, s. 7, s. 8), not unlike those more anterior. The sternum of the ninth segment of the male abdomen is a single small heart-shaped sclerite (fig. 24, s. 9), which articulates laterad with the claspers and caudad at its apex with the oedagus. In the female the immense ovipositor extends so far cephalad that the sternum of the seventh somite (fig. 28, s. 7) is deeply indented, its caudal border forming an arc cephalad which almost divides the sternum into two plates at its median plane. The sternum of the eighth somite in the female (fig. 28, s. 8) is represented by two laterally situated plates, each articulating with a gonapophysis of the pair belonging to that somite (fig. 28, gn. 8). In the ninth somite also the sternum is divided into two parts, each having the outline of a Roman lamp. Each of these plates (fig. 28, s. 9) is covered by the pleuron of its respective side and has articulations with two gonapophyses (gn. 9 and gn. 9'), one at its cephalic end and one at its caudal end.

In parasitized males, although the genitalia are not produced cephalad, the sternum of the eighth somite is often almost divided into two plates by a deep notch from its caudal border (fig. 27,

s. 8). The sternum of the ninth segment, instead of being a single heart-shaped plate, is represented by two minute sclerites, each placed laterad against the clasper of its corresponding side and about equidistant from the ends of this organ. It would be difficult to explain these changes in infected males as being due to mechanical necessity caused by alterations of the gonapophyses, and they can only be understood, I believe, in looking at them as a partial assumption of female characteristics.

The sterna of parasitized females show the following changes: that of the seventh segment bears merely a notch in the median plane running cephalad from its caudal border; that of the eighth remains a single sclerite, being not quite bisected by the gonapophyses (fig. 31, s. 7, s. 8) which are greatly shortened by the action of the parasites. The ninth segment presents the sternum in two sclerites, retaining their original position and articulations, but being somewhat larger than normal and less well chitinized.

A distinct sexual difference is found in the tergum and pleura of the ninth abdominal segment. In the male (fig. 24, *t. 9, p. 9*) the tergum and pleura of this segment are separate sclerites which are united by a thin, pliable, chitinous membrane. The pleuron is almost semicircular and projects caudad beyond the tergum. In the female (fig. 28, *t. 9, p. 9*) these sclerites are much larger and quite different in form from those of the male. They are fused into one, the suture on each side being visible as a curved line passing from the anal tube to the lower border of the eighth tergum. The integument on each side of this suture is of a distinct character; the dorsal portion being similar to that of the other terga, whereas the ventral portion is beset with long hairs. The pleuron, unlike the semicircular sclerite of the male, is drawn into a long plate, extending almost the entire length of the external genitalia (fig. 29, *p. 9*).

In parasitized males (figs. 26 and 27, *t. 9, p. 9*) the tergum and pleura fuse as in the female. Moreover, both plates increase in length, the pleura lose their semicircular form and fail to project caudad beyond the tergum. Thus all the male characteristics are lost and an approach to the female condition takes place.

In parasitized females (figs. 30 and 31, *t. 9, p. 9*) no qualitative change occurs; the tergum remains practically normal and the pleura decrease in length, accommodating themselves to the shortened gonapophyses.

Summing up the changes in the extragenital abdominal characteristics in parasitized males, we find that the abdomen increases in size; that the pleura become flat and ventral in position and in cross-section the abdomen is similar to that of the female; that the hard dark brown integument becomes thin and pliable and lightly pigmented; that the patterns formed by the minute spines on the terga and sterna assume the arrangements characteristic of the female; that the sternum of the eighth abdominal segment is almost divided into two plates, while that of the ninth often separates completely, forming two minute, lateral sclerites; that the tergum and pleura of the ninth segment fuse, increase in length, and partially assume the form of the corresponding sclerites of the female.

In the females the parasites do not cause the abdomen to increase in size or change its form in cross-section. However, the integument becomes thinner and there is some loss in pigmentation. Since in the female the pigmentation is normally much less intense than in the male and the cuticula more pliable, these changes are not nearly so radical as those in the male. The arrangement of the minute hairs on the abdominal sclerites remains unaltered in the female. The sternum of the seventh abdominal segment is not so deeply notched at its caudal border and that of the eighth segment only partially divided into two sclerites by the ovipositor, which is much shortened. The form of the tergum of the ninth segment remains unchanged, while the pleura decrease in length.

D. The genital appendages

As has been intimated in the foregoing pages, the external genitalia suffer a considerable reduction in size in parasitized individuals of both sexes. This is quite in contrast in the male to the reaction of the extragenital secondary sexual characteris-

tics. Since the head, thorax, and abdomen of parasitized males show such a decided change toward the female condition, it was of course puzzling to understand why the genital appendages showed not the slightest tendency toward such a transformation. A study of the structure of the adult gonapophyses of both sexes and their mode of origin was undertaken. The results of this study not only justify the classification of sexual characteristics used in this paper, but also show why the two categories of secondary sexual characteristics behave differently under the effects of parasitism.

The external genitalia consist of three pairs of appendages in both sexes. In the male they are terminal, located on the last complete abdominal segment, the ninth. In the female they are produced cephalad on the ventral surface, reaching into the indentation of the seventh sternum. They are, however, connected entirely with the sclerites of the eighth and ninth abdominal somites.²

The male genitalia (figs. 24 and 25) consist of a pair of ventral valves, a pair of styles or claspers, and an unpaired oedagus. The ventral valves (fig. 24, *v.*) are flattened sclerites united in the median plane for more than half their length, and produced caudad into a pair of free, narrow appendages, composed of two layers of chitinous cuticula closely approximated and enveloping the hypodermis which produced them. The claspers are strong chitinous rods, each produced into a hook laterad to the oedagus (figs. 24 and 25, *cl.*). Each articulates with the side of the heart-shaped sternum of the ninth segment and extends

² The terms used in reference to the genital appendages are merely descriptive and bear no phylogenetic implications. From a study of a more primitive homopteran, such as the ordinary *Cicada linnei* Grossb., one may see that the gonapophyses originally arose from the eighth, ninth, and tenth abdominal somites. This is very clearly seen in the female, although a trifle obscured in the male, where the appendages are so modified and specialized that they are of great use to the systematist in a study of the Cicadidae. It is the writer's belief that in the membracids the gonapophyses originated from the primitive eighth, ninth, and tenth abdominal appendages, even though in the male they all seem to arise from the ninth segment. A careful study of the embryonic stages might reveal a migration and persistence of the cells of the limb buds of the segments in question.

cephalad within the abdomen half-way through the eighth somite. To this internal portion muscles are attached which move these appendages. The oedagus is a heavily chitinized, tubular organ containing the penis. Its proximal end articulates with the caudal tip of the ninth sternum. The oedagus bends sharply dorsad and comes into close approximation with the anal tube (figs. 24 and 25, *oe.*).

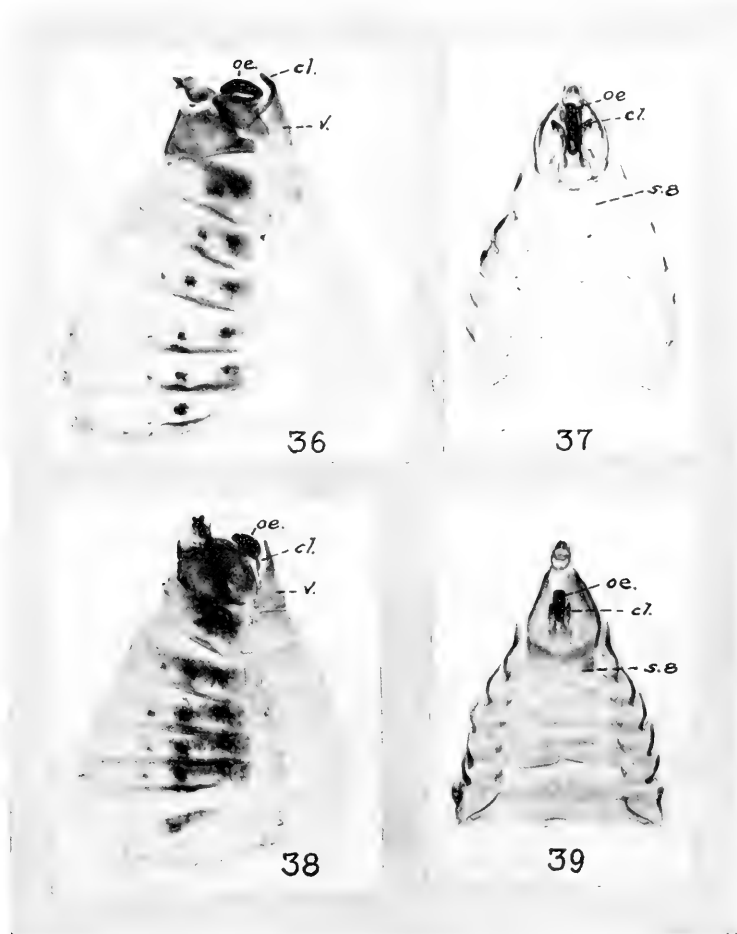
The female external genitalia are seen in figures 28 and 29. The appendages of the eighth segment are two sharply pointed plates, each articulating with the lateral remnant of the eighth sternum (fig. 28, *gn.* 8). They form a strong, closely fitting sheath about the ovipositor, being interlocked along the median plane by a sort of dove-tail union. The anterior appendages of the ninth segment form a tube, the ovipositor, open along its ventral border at both its proximal and distal ends (fig. 28, *gn.* 9). Its proximal portion consists of two strong, chitinous rods which diverge and articulate laterad with the two small sclerites representing the ninth sternum. The distal third of the ovipositor bears three pairs of well-defined teeth along its dorsal border. The third pair of gonapophyses, the posterior pair of the ninth segment, are flattened sclerites, laterally placed, partly covering the ovipositor and its sheath (fig. 28, *gn.* 9'). Each articulates with the caudal portion of the ninth sternum of its respective side.

In parasitized males the oedagus and claspers are greatly reduced in size and are located on the ventral portion of the enlarged eighth somite (figs. 26 and 27). The oedagus is shortened to form a stout tube, and the claspers do not reach even to the base of the ninth segment, but retain quite well their original form. The ventral valves show much less reduction in size than the oedagus or clasper. They, however, present many irregularities in that the free caudal projections are often misshapen and of unequal length. On the average, the ventral valves are reduced only 12 per cent and the maximum reduction found was 24 per cent of the normal length. Their reduction is not as constant as that of the other male genital appendages, for in males, otherwise greatly altered, they may at times be practically normal in length (fig. 26, *v.*).

If, now, we examine parasitized males, which have retained the more striking tertiary sexual characteristics of color, form, and size (figs. 38 and 39), we find that they, too, generally have much reduced genitalia. Occasionally, male colored individuals with parasites present only a partial reduction of the gonapophyses and these most probably represent those parasitized shortly before their moult to the adult form (figs. 36 and 37). The above facts show that the genital appendages are very sensitive to the influence of *Aphelopus*, being reduced even in individuals in which the effect of the parasites was not sufficient to alter the pigmentation of the pronotum or face.

In parasitized females, all three pairs of genital appendages show a great reduction in size (figs. 30 and 31). They are sometimes weakly chitinized, bent, and misshapen. The ovipositor (fig. 30, *gn. 9*) does not always form a tube, but consists of two separate plates diverging distally. All three pairs of appendages are reduced proportionately, there being no one pair as little affected as the ventral valves of the male genitalia.

The above facts show that, although there is a decided reaction of the external genitalia due to parasitism, yet there is not the slightest tendency of these appendages to assume the characteristics of the opposite sex. They are, therefore, entirely different in the male from the extragenital secondary characters in their behavior. The reason for this is to be sought in a consideration of the origin and development of these two categories of characteristics. The extragenital secondary or tertiary sexual characteristics, such as color of pronotum and face, arrangement of spines on the abdominal sclerites, and many others, arise during the fifth nymphal instar and make their first appearance in the adult Thelia. If the nymph be a male and contain well-grown *Aphelopus* larvae, the resulting adult will exhibit female extragenital sexual characteristics; but the genitalia, though greatly reduced in size, will unquestionably remain male in character, because these appendages did not arise in the fifth nymphal instar, but were laid down early in ontogeny, either before the parasites were present or while the parasites were minute and incapable of exerting any marked influence.



Figs. 36 to 39 Abdomens of parasitized males which still retained almost normal coloration of pronotum. Compare with figures 24 to 27. Figure 36, lateral view, abdomen compressed, only partial reduction of gonapophyses. Figure 37, ventral view, abdomen not compressed, partial reduction of gonapophyses. Figure 38, lateral view, abdomen compressed, extreme reduction of gonapophyses. Figure 39, ventral view, abdomen not compressed, extreme reduction of gonapophyses. *oe.*, oedagus; *cl.*, elasper or style; *v.*, ventral valve; *s. 8*, sternum of eighth abdominal somite. $\times 9$.

Many descriptions of membracid nymphs have been written and a detailed account of the five nymphal instars of *Thelia* has recently been published (Funkhouser, '15), but no mention is made of characteristics which may be used to distinguish the sexes. Neither in coloration, nor in the form of the integument, nor in the thoracic appendages is any clew given to the sex of the nymph. In the fifth instar the female abdomen is noticeably larger and wider than the male abdomen; but an infallible test for the sex of the nymph is to be found by examining the ventral surface of the eighth and ninth abdominal segments. The genitalia are so well developed in the nymphs and are so distinctly different in male and female that, as early as the third instar, one can determine the sex by an examination of the ventral surface of the eighth and ninth somites with a hand lens. Figures 40, 41, and 42 show the form of the genitalia in male nymphs of the third, fourth, and fifth instars, respectively, whereas the corresponding stages in the female are shown in figures 44, 45, and 46. The sexes are easily recognizable, not only by the form and position of the appendages, but also by the pigmentation of the eighth sternum.

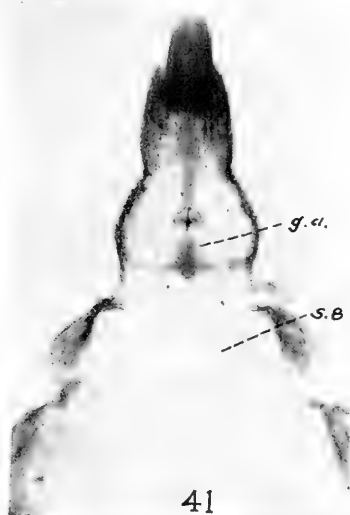
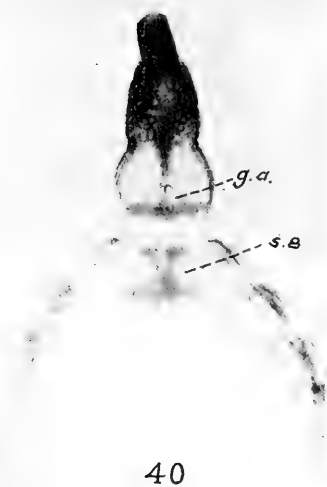
Examining the characteristics of the three stages of the male represented in figures 40, 41, and 42, we find that the genitalia arise in a triangular-shaped area on the ninth abdominal segment. Small chitinous pockets pointing cephalad contain then hypodermal cells which produce the genitalia. In each instar

Fig. 40 Abdomen of male third nymphal instar, ventral view, caudal portion showing genital area (*g. a.*) and characteristic pigmentation of sternum of eighth somite (*s. 8*). $\times 26.2$.

Fig. 41 Abdomen of male fourth nymphal instar, caudal segments, ventral view, showing inner (dorsal) pockets as smaller pair, and outer (ventral) pockets of genital area. Medial partition in each pair. $\times 26.2$.

Fig. 42 Abdomen of male fifth nymphal instar, ventral view of entire abdomen. Inner pockets now three in number, median and two lateral; outer pockets still right and left of median partition. Pigment confined to ends of pockets and partitions. $\times 9.7$.

Fig. 43 Abdomen of parasitized male fifth nymphal instar, ventral view of entire abdomen, showing extreme reduction of inner pickets of genital area due to action of parasites. The holes in the sterna were made by escaping eruciform larvae, which left their exuviae (those of megagnathic stage) at puncture holes. $\times 9.7$.



these cells within the pockets produce the appendages of the next stage. In molting the newly formed appendages are withdrawn from these pockets cephalad, and the soft, wrinkled integument unfolds to produce the larger gonapophyses. Thus there is a gradual growth of the external genitalia. In the male third instar there are two pairs of pockets, one placed dorsally to the other on the ninth segment. Each pair is separated into its right and left components by a median chitinous partition extending cephalad from the apex of the triangular genital area. The inner pair of pockets is smaller than the pair more ventrally placed. There is no evidence for any appendage arising from the eighth somite in the male. The ninth segment has a band of melanic pigment about its anterior portion and this pigmented band is slightly more intense where it crosses the genital area. The sternum of the eighth segment bears a pigmentation characteristic of this stage and of the male sex. There is a light medium stripe and a light patch extending from each side toward the median plane, and this is quite different from the pattern on the eighth sternum of the female third instar. In the fourth instar (fig. 41), the triangular genital area increases considerably in size and the two pairs of chitinous pockets are more easily seen at its apex. The pair more dorsally placed does not extend quite so far caudad as the larger ventral pockets. The pigmented ring of the ninth segment remains and broadens out on the genital area, while the melanin on the eighth sternum retains its former pattern, but is less intense. In the fifth instar (fig. 42), the ventral pockets which produce the ventral valves of the adult retain the form seen in the previous stage, a median partition separating the right from the left, but the dorsal pair change greatly. Instead of a median chitinous partition, there are two lamellae, which divide the pocket into three subdivisions: a larger median compartment and two smaller lateral compartments. In the median pocket the oedagus develops, and in the lateral pockets, the claspers. The melanic pigment is restricted to the caudal end of the genital area.

Turning now to the development of the ovipositing apparatus, we find in the third instar (fig. 44) a pair of darkly pigmented,

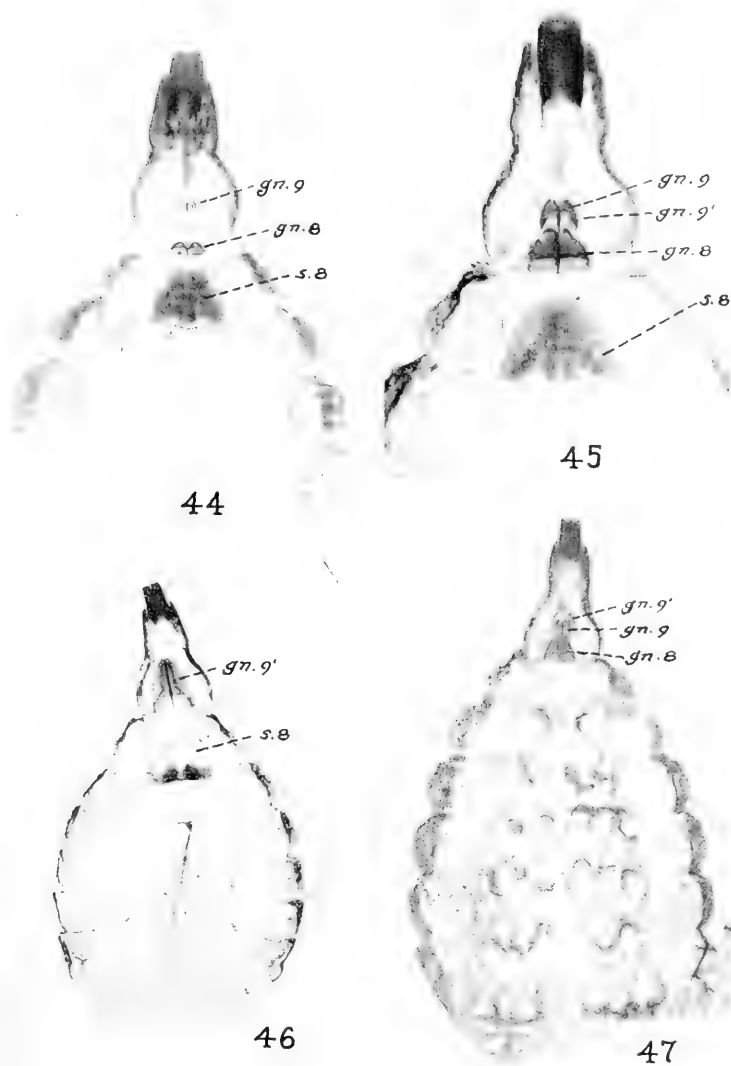


Fig. 44 Abdomen of female third nymphal instar, ventral view of caudal portion, showing gonapophyses of eighth (*gn. 8*) and ninth (*gn. 9*) segments, and pigmentation characteristic of sternum of eighth somite (*s. 8*). $\times 26.2$.

Fig. 45 Abdomen of female fourth nymphal instar, ventral view of caudal portion. All three pairs of gonapophyses represented: one pair of the eighth (*gn. 8*), and two pairs on the ninth segment (*gn. 9*, and *gn. 9'*). The adult appendages which these form are shown with similar designations in figures 28 and 29. $\times 26.2$.

Fig. 46 Abdomen of female fifth nymphal instar, ventral view of entire abdomen, showing growth caudad of *gn. 8*, which now cover and extend beyond *gn. 9*. *gn. 9'* now partly overlap the two median pairs of gonapophyses. $\times 9.7$.

Fig. 47 Abdomen of parasitized female fifth nymphal instar, from which larvae have emerged. Retardation of gonapophyses, which remain similar to those of fourth instar. $\times 9.7$.

chitinous pockets arising from the caudal border of the eighth sternum. The presence of these tell us immediately that the nymph is a female, as does also the pigmented area on the eighth sternum anterior to these pockets. On the ninth segment is a pair of narrow pockets more deeply pigmented at their posterior border. In the fourth instar the three pairs of nymphal appendages which form the three pairs of adult gonapophyses can be most plainly seen (fig. 45).

The pair arising from the eighth somite, which in the adult form the sheath covering the ovipositor, have grown considerably caudad, partly overlapping the median and anterior pair of the ninth segment which eventually produce the ovipositor itself. Lateral to the anterior pair of the ninth segment are two lightly pigmented, slender, curved pockets, and in these the posterior gonapophyses of the ninth segment are produced. The pigmented area of the eighth sternum is similar to that of the third instar. The fifth instar (fig. 46) shows a considerable growth and closer approximation of all three pairs of appendages. Those of the eighth segment extend caudad beyond the narrower pair of pockets which form the ovipositor. The posterior appendages of the ninth segment now form broad lateral pockets, partly overlapping the two median pairs. As the adult appendages develop they are seen through the nymphal cuticula, the most anterior gonapophyses extending from the pigmented cephalic border of the eighth segment beyond the tip of the ovipositor.

Thus we see that the genitalia develop quite differently in the two sexes and are determined quite early in ontogeny. The above description goes back merely to the third instar, at which stage one can distinguish the sexes with ease from surface views; but long before this the anlage for the gonapophyses are formed. Careful examination of whole mounts of second instars also reveals the differences: the form of the genital area on the ninth segment is broad and in the form of a semicircle in the male, whereas in the female it is much narrower and resembles that of the third instar. In the female one can also see the beginnings of the gonapophyses of the eighth segment as two minute chit-

inous elevations on the caudal border of the eighth sternum. Going back still farther to the first instar, longitudinal sections³ show thickenings of the hypodermis corresponding to the appendages seen in surface views of later stages, and, since the gonads are already differentiated into minute ovaries or testes, one knows positively the sex with which he is dealing.

In considering the effects of the parasites on the gonapophyses, it might be inquired whether or not these appendages are modified in their nymphal form by the larvae of *Aphelopus*. As was stated on page 552, nymphs stung during their first or second instars do not become adults, for the internal larvae reach maturity in the fifth instar of the host and emerge, thereby killing the *Thelia*. Two such nymphs from which the larvae have emerged are shown in figures 43 and 47. In the male (fig. 43) there are two changes in the nymphal genitalia. The dorsal pockets suffer a great reduction in size and remain in part similar to those of the fourth instar in that the median partition still persists. The two pockets are each again subdivided by a partition corresponding to those of the normal fifth instar, but these are very small and run parallel to the long axis of the animal rather than at an angle toward the median plane, as in normal nymphs. The condition is, therefore, intermediate between the fifth and fourth instars, for the median partition is characteristic of the fourth instar and is not present in the fifth instar, whereas the lateral partitions are characteristic of the fifth instar and not present in normal fourth instars. The persistence of a characteristic of the fourth instar indicates retardation of development. In the parasitized female nymph (fig. 47) not only are the nymphal genitalia reduced in size, but their form corresponds exactly to that of the normal fourth instar, each pair of gonapophyses remaining distinct and easily differentiated. After having ascertained that during the fourth

³ At the suggestion of the author, Mr. E. D. Churchill made a histological and gross study of the development of both external and internal genitalia of the membracid *Vanduzea arquata* (Say). In this he traced the gonapophyses from the first instar in histological sections. His work substantiates exactly what has been found true of *Thelia*.

instar the parasites exert an influence upon the host sufficient to change the appendages at the succeeding molt, an attempt was made to see if this could be carried back to still earlier stages. Accordingly, a number of male and female nymphs of fourth instar each containing parasites of maximum size for that stage (0.75 mm.) were prepared and mounted similar to the normal individuals shown in figures 41 and 45. Careful camera-lucida drawings were then made of twenty individuals, ten parasitized and ten normal. The width and length of the nymphal appendages were measured on the drawings, and in neither sex could any alteration from the normal in the size or form of the appendages or in the pigmentation of the integument be found. From this we may infer that in the third instar the parasites are still too minute to exert any influence upon the cells producing the integument and appendages of the fourth instar. Since the genital appendages are laid down even before the third instar, we can see that the parasites could in no way interfere with the formation of the anlage of the gonapophyses. Only during the fourth and fifth instars do the parasites affect the genitalia, and this effect appears in the retardation of development and reduction in size found in the fifth nymphal instar and the great reduction in size in the adult. Even minute parasites, still spherical embryos just becoming separate individuals, cause the adult genitalia to be reduced. This is probably due to the fact that the greatest step in the formation of the gonapophyses comes in the development of the adult from the fifth nymphal instar. That the genitalia are reduced in size but retain their general form in parasitized individuals is probably to be ascribed to their history.

It is generally conceded that organs formed early in ontogeny are phylogenetically older than those appearing late in development. The extragenital secondary sexual characteristics which arise during the fifth instar certainly belong to the species. The genital appendages are relatively older and appear early in ontogeny. In the later stages of development various specific modifications may slightly alter the gonapophyses. Systematists have shown that the Membracidae possess a relatively

ancient type of genitalia found in practically all primitive homopterans. Funkhouser ('17) considers that the family Membracidae is the lowest of the Homoptera with the exception of the Cicadidae. One of his four reasons for this assumption is that in the Membracidae "the genital organs are simple. Little progress has been made in developing these structures from an ancient type." Considering, then, that the gonapophyses of Thelia are still of the same type as that established when the order Homoptera evolved in the distant past, we see why, under the effects of parasitism, they remained more constant than did the extragenital sexual differences. The formation and sexual differentiation of these appendages are started before the parasites are present. The mechanism once under way is not reversed by parasitism, but the resultant products are greatly reduced in size and suffer, as will be shown, the loss of certain adult characteristics specific for *Thelia bimaculata*.

Giard ('89) pointed out that parasitized individuals of *Typhlocyba* showed a reduction of the external genitalia in both sexes. The male of *Typhlocyba hippocastani* has a very complicated oedagus produced into eight branches at its distal end, and this organ is specific for *hippocastani*, distinguishing it nicely from *T. douglasi*. Males parasitized by *Atelenevra* show a reduction of the branches of the oedagus to six, four, or three. Thus the specific character of the oedagus is 'profoundly modified,' so that parasitized *hippocastani* may be confused with *Typhlocyba rosae* L. or *Typhlocyba lethierryi* J. Edw.

The specific characteristics of the gonapophyses first appear in the final molt, and they are probably recent from a phylogenetic standpoint. As other specific characteristics, one would expect that they would fail to develop or develop but partially in parasitized individuals. Thus a comparative study of the gonapophyses of *Thelia bimaculata* Fabr. and its nearest available relatives was undertaken, to ascertain if *bimaculata* possessed any specializations which might be looked upon as specific modifications. Through the generosity of Dr. E. D. Ball various membracids of the tribe Telamonini were made available, and these fortunately included the rare *Thelia uhleri* Stål. Normal

individuals of both sexes belonging to the following species were prepared and mounted in balsam as was done earlier for *Thelia bimaculata* (figs. 24 and 28): *Thelia uhleri* Stål, *Glossonotus acuminatus* Fabr., *Glossonotus godingi* Van D., *Telamona querci* Fitch, *Telamona reclivata* Fitch, *Archasia belfragei* Stål, and *Carynota mera* Say.

The one outstanding characteristic of the male genitalia of *Thelia bimaculata* which distinguishes it from the others studied is the shape and length of the oedagus. All the *Telamonini* have the oedagus bent sharply dorsad toward the anal tube. At the bend, in the forms above named with the exception of *Thelia bimaculata*, the oedagus is narrowed and becomes thicker and bulbous toward the distal end. Viewed in profile, the inner surface of the portion dorsad to the bend is practically a straight line, whereas the outer or free surface is greatly curved. *Thelia uhleri* Stål, the nearest relative to *Thelia bimaculata* Fabr., also possesses a bulbous oedagus narrowed at the bend dorsad; but *T. bimaculata* has a slender almost tubular oedagus, not bulbous at its distal end or narrowed at the bend dorsad. Its inner surface viewed in profile is not a straight line, but is curved so as to be practically parallel to the outer or free surface. In comparison to its diameter, the oedagus of *Thelia bimaculata* is longer than in any of the other forms studied, and its distal third bends cephalad as well as dorsad. In parasitized individuals the oedagus is reduced in length much more than in diameter. The inner edge shows a decrease in its curvature so that the thick bulbous form found in the other *Telamonini* becomes approximated in the reduced organ. Thus the specific form of the oedagus so characteristic for *Thelia bimaculata* is lost.

The female gonapophyses of the various *Telamonini* available were likewise studied. Of all the forms *Thelia bimaculata* had the bluntest, most rounded tip at the distal end of its ovipositor. The ends were found to be strongly chitinized and reinforced by longitudinal ribs. In all the forms, with the exception of *Thelia bimaculata*, near the tip of the ovipositor on its ventral border there is a thin translucent area. This is directly anterior to a chitinous rib which runs diagonally on

each side of the ovipositor from a median rib toward the ventral border. *Thelia uhleri* has a pointed ovipositor with a translucent area behind a diagonal rib near the tip. This seems to be a general characteristic of the Telamonini studied. Ovipositors of parasitized *Thelia* are not only reduced in size, but they are more pointed at their distal ends than those of normal individuals. The chitinous ribs are reduced in size, and one may easily discern a thinner area near the tip on the ventral border.

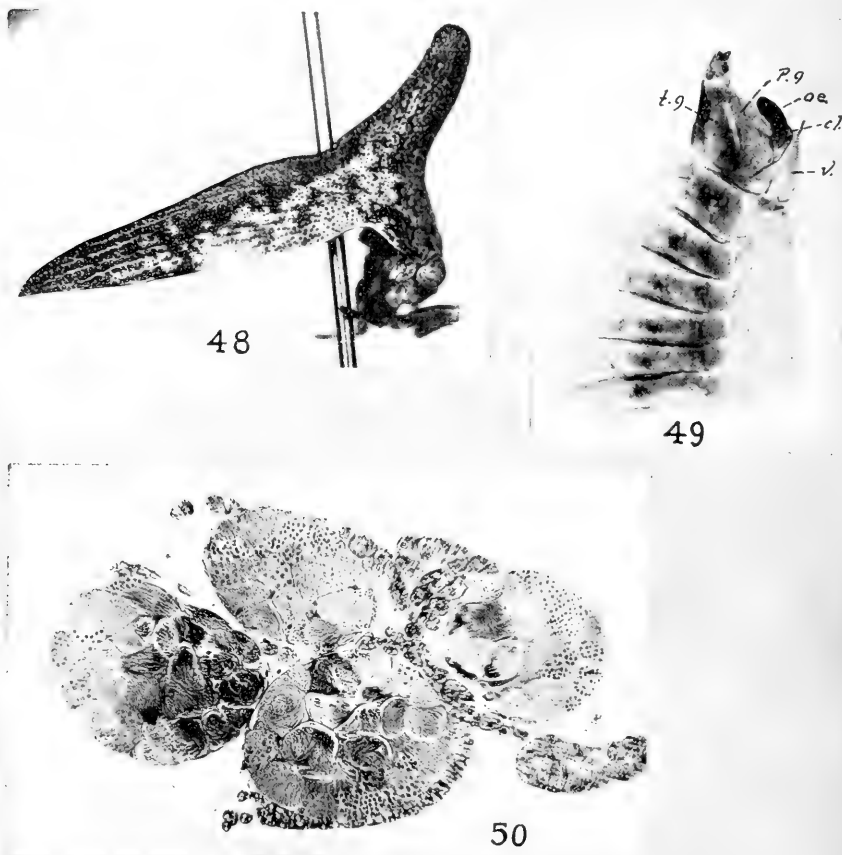
Thus both oedagus and ovipositor lose through parasitism characteristics specific for *Thelia bimaculata*. These specific characteristics of the genitalia must be recent acquisitions in the genetic make-up of this highly specialized form. They merely modify and add details to the gonapophyses, organs long established and rather stable in most homopterans. Whereas the genitalia of parasitized individuals retained the general form found in the tribe Telamonini, they lost their specific characteristics through the action of the parasites.

7. AN ACCOUNT OF TWO SIGNIFICANT INDIVIDUALS

Two extraordinary individuals, one captured in the summer of 1916 and the other in the summer of 1917, throw considerable light on the relation of the gonads to the soma in *Thelia*, and are too important to omit even in this paper which deals mainly with the effect of the parasites upon external features of the host.

The first individual was an adult parasitized male. The parasites were of medium size, measuring 0.75 mm. from the post-cephalic region to the posterior end of the abdomen. Their mouth parts were not chitinized and the curved bodies of the parasites were enclosed in nutritive envelopes. However, the larvae had already exerted a marked influence on the host. The pronotum (fig. 48) showed considerable change in coloration toward the female condition. The bright yellow was absent from the vitta, and the melanic pigment over the rest of the pronotum was no longer uniform and encroached upon the clear chitin of the vitta. The length of the pronotum was 11.90 mm.,

which is 0.35 mm. greater than the average length in males. The fore wing showed even a more marked increase in length, being 9 mm. long, which is nearer the average length in normal



Figs. 48 to 50 Views of a parasitized male which contained one full-sized testis and showed somatic changes toward the female condition. Figure 48, pronotum. $\times 6$. Figure 49, abdomen, lateral view of left half compressed under cover-glass; *oe.*, oedagus; *cl.*, clasper; *p. 9*, pleuron of ninth segment; *t. 9*, tergum of ninth segment. $\times 9.7$. Figure 50, four tubules of testis as seen in section. $\times 84$.

females (9.78 mm.) than the average length in normal males (8.13 mm.). The pattern of the face also showed a decided loss of male characteristics and an assumption of those of the female.

Turning now to the abdomen (fig. 49), we find there also striking changes. The terga form a sharp ventrolateral angle where they join the pleura and the cuticula shows a marked reduction of melanin pigment and has become more pliable. Examined microscopically, the terga exhibited the arrangement of the minute spines in the pattern characteristic of the female (figs. 32 to 35). The tergum and pleura of the ninth abdominal segments fused together and became longer than those of normal males. The oedagus and claspers were greatly reduced in size.

The most interesting feature about the male *Thelia* described above is that in the right half of the abdomen there was located an entire testis, normal in size. This was immediately removed and placed in Bouin's fluid, drawn with the aid of a camera lucida to obtain its exact dimensions, and then imbedded and sectioned. All stages of active spermatogenesis were found. There were spermatogonia quiescent and in mitosis, spermatocytes in growth and maturation, spermatids undergoing transformation, and mature spermatozoa in great numbers. Figure 50 is a photograph of four of the tubules, and, even at the comparatively low magnification used, one may distinguish the cysts of spermatozoa with their deeply staining heads lying side by side. The mitoses found in this testis were in every way normal and the large, lagging, unpaired x-chromosome is as noticeable as in any normal first spermatocyte division (fig. 51, *e* and *f*).

The testis above described was the largest ever found in a parasitized *Thelia* which showed marked somatic changes. Other testes have been taken from altered males, but these invariably showed many abnormal mitoses, many stages of fatty degeneration, and broken-down cysts devoid of spermatozoa. These have been sectioned and studied and will be reported upon at a future date. The important fact to be noted about this male is that, in spite of the presence of a normal testis, the parasites exerted a marked influence on the developing soma. This

is evidence that the effect of the parasites is not accomplished through the destruction of the testes of the host (castration parasitaire), but that the parasites have a direct effect upon the developing tissues.

The second significant individual was a nymph of the fourth instar. The author was dissecting a large number of nymphs

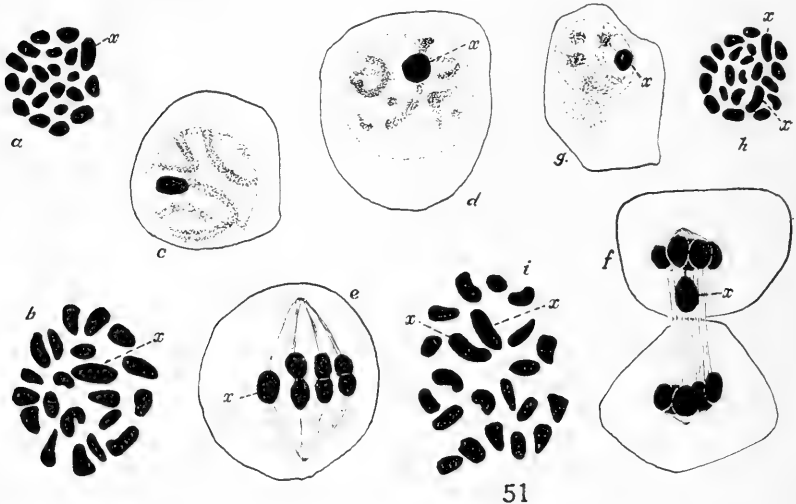


Fig. 51 Cells from normal *Thelia* gonads and soma. *a*, spermatogonium, 21 chromosomes including one x-chromosome, the largest of the group (*x*); *b*, somatic cell from developing external genitalia, removed from fifth nymphal instar and stained with acetic-carmin, 21 chromosomes, one large x-chromosome; *c* and *d*, primary spermatocytes showing large x-chromosome; *e*, primary spermatocyte, lateral view of first maturation spindle showing large unpaired x-chromosome; *f*, late anaphase of first spermatocyte division; *g*, spermatid which has received x-chromosome; *h*, oögonium, 22 chromosomes including two x-chromosomes (the largest pair); *i*, somatic cell from developing external genitalia, acetic carmine, 22 chromosomes. $\times 1980$.

of this stage when he was surprised, upon opening the abdomen of a normal-appearing female, to find that a pair of testes, instead of ovaries, were present. The testes were quickly removed, one preserved in Bouin's fluid and the other prepared for immediate observation in Schneider's aceto-carmin. The Bouin material sectioned (fig. 53) proved far more valuable than

the freshly stained material. The body of the nymph was preserved in Gilson's fluid and later sectioned, with the exception of the caudal end of the abdomen, which was prepared for whole mount (fig. 52).

The structure of the gonads of this individual was compared with that of testes and ovaries of normal fourth instars. The normal testis is composed of tubules which are almost spherical and are bound together by their efferent ducts which unite centrally to form the chief duct. This duct runs in the direction of the long axis of the testis, which is roughly an ellipsoid in form. The testis from surface view looks like a bunch of grapes. Each tubule is composed of a number of well-differentiated cysts, marked off by definite walls. Each cyst is filled with spermatogonia or spermatocytes, the cells in any one cyst being approximately in the same stage of growth or mitosis. The ovary presents a very different structure. The most conspicuous portion consists of terminal chambers, elliptical in longitudinal section and placed side by side in a dorsoventral plane of the abdomen. From the anterior end of each terminal chamber runs a terminal filament, and from the posterior end, an ovarian tubule which later contains the large oöcytes. The terminal filaments of each ovary converge and form a single support for the gonad, and the tubules likewise converge caudad to form an oviduct. A section of the terminal chamber shows a wall of tall epithelial cells with clear cytoplasm. These cells are unlike the flattened epithelial cells covering the testicular tubules. In the region of the attachment of the terminal filament, one encounters oogonia in various phases of mitosis, and the rest of the chamber is filled with nurse cells and small oöcytes preparatory to growth. There are no subdivisions of the chamber into cysts. Thus, macroscopically and microscopically, the gonads of the two sexes are so distinctly different that they are not easily confused. That the gonads of the peculiar nymph under discussion were testes, there is not the least doubt. Certain peculiarities of these testes will be noted later.

We are, therefore, considering an individual with female soma and male germ glands. As already noted on page 586 (fig. 45),

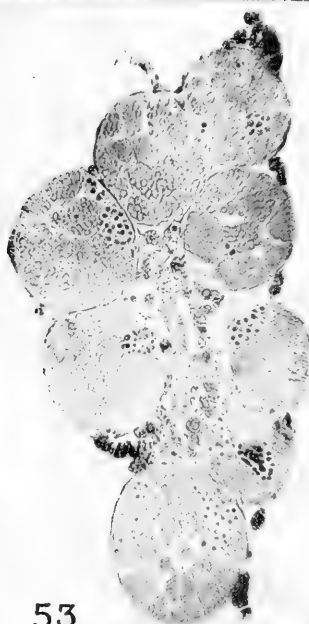
the three pairs of appendages which form the adult external genitalia may be seen on the ventral side of the eighth and ninth abdominal segments of female fourth instars. These appendages and the pigmented area of the eighth abdominal sternum comprise the external sexual characteristics of the nymph. When compared by means of camera-lucida drawings with normal female individuals similarly mounted, the external genitalia and pigmented area of the eighth sternum of this anomalous nymph (fig. 52) were not in any way abnormal. This is a distinct proof that in *Thelia* the testes do not pour forth a secretion which influences the developing soma, or, in other words, the soma is independent of the gonads in its development. And we may also conclude from this individual that the development of the gonad is to a large extent independent of the sex of the body in which it is found.

An inquiry into the cause of the development of testes in this peculiar nymph with female soma was next undertaken. A cytological study of the testis proved most instructive. Spermatogonia in mitosis were very abundant and handsomely preserved so that the chromosomes stood out with diagrammatic clearness. Eight of the most favorably situated metaphase plates are shown in figure 54. These represented about one-fourth of the number of spermatogonia in which the chromosomes could be accurately counted. The drawings were all made with a camera lucida, and in no cases are they reconstructions from two or more sections. Neither are the chromosomes shifted from their original positions. As is easily seen in figure 54, these cells are true spermatogonia containing twenty-one chromosomes, the largest being the unpaired x-chromosome. For comparison, the chromosomes of soma and germ-plasm of

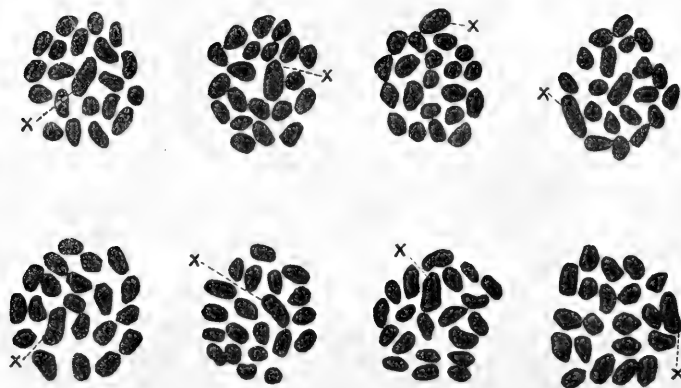
Figs. 52 to 54 Abdomen, testis, and spermatogonia from anomalous fourth instar with female soma and male gonads. Figure 52, ventral view of caudal portion of abdomen, showing external genitalia and pigmentation of eighth abdominal sternum; compare with figure 45. $\times 30.6$ Figure 53, longitudinal section of one of the testes, showing eight tubules, each divided into cysts. $\times 140$. Figure 54, eight spermatogonia from testis, a section of which is shown in figure 53. Each spermatogonia contains 21 chromosomes, the largest being the unpaired x-chromosome (x); compare with figure 51, *a*. $\times 2200$.



52



53



54

normal *Thelia* are shown in figure 51, most of the figures being reproduced from a former paper (Kornhauser, '14). Figures 51, *a*, and 51, *b*, show the diploid male group taken from testis and developing gonapophyses, respectively. These show twenty-one chromosomes, and the largest of each group is the unpaired x-chromosome. Diploid groups from the ovary and soma of the female are shown in figures 51, *h*, and 51, *i*, and exhibit twenty-two chromosomes, which include two large x-chromosomes.

In this anomalous nymph the testes contained typical spermatogonia, and, since these cells are direct descendants of the primary germ cell which gave rise to the gonads, it was most probably the chromatic make-up of this primordial cell which caused the development of testes instead of ovaries. It is the author's opinion that the zygote from which the nymph arose was female and that an abnormal mitosis gave rise to a primordial germ cell lacking one of the x-chromosomes. Having the male diploid group of chromosomes, this cell proceeded to form male gonads even though nourished and enclosed within the body of a female. An attempt was made to analyze the chromatic composition of the soma of this nymph, but on the whole the cells were not favorable for accurate counts. No metaphase plates were clear enough to count. Two good pro-phases indicated the presence of twenty-two chromosomes in body cells, but counts of such cells cannot be regarded as entirely satisfactory. Nevertheless, since the soma was purely female, we may feel fairly certain that the cells which composed it had the female chromosome complex, just as the gonads which were male had the male chromosome complex.

Meisenheimer ('09) and Kopeć ('11) maintained that ovaries transplanted into castrated males produced small ova because there was not sufficient room for development, and did not ascribe any influence to the metabolism of the soma in which they were placed. Their view seems rather extreme, and in the case of the testes of the nymph with female soma we find quite an effect produced by the soma on the size of the gonads. In *Thelia*, as in most insects, the testes develop much more rapidly

in ontogeny than do the ovaries, so that when the imaginal form is reached the testes have already reached their maximum size and contain mature sperm, whereas the ovaries are still very immature. This same discrepancy in rate of development may also be noted in the various nymphal instars. Thus in the fourth instar of *Thelia* the testicular tubules average 0.2 mm. in length, whereas the terminal chambers of the ovaries average 0.1 mm. in length. In the testis of the anomalous nymph, the tubules were on the average 0.15 mm. long, making the testes quite noticeably undersized. This would indicate that the rapid growth of the testes in normal males is not due entirely to the properties of the germ cells, but that the soma which provides the nutriment contributes in no small measure to the growth of these cells. In this nymph with female soma the testes did not get all the nourishment they required, but probably they did get more material for growth than is ordinarily supplied to an ovary at this stage. The lack of sufficient nutriment evinced itself in the presence of many cysts of degenerating spermatogonia, such as may often be found in mature testes, but not in normal testes of nymphs. Previously (Kornhauser, '14, p. 251) it was suggested that the degeneration of cysts of spermatogonia, found so often just at the close of the period of multiplication in testicular tubules, was due to the lack of sufficient cytoplasmic materials, and the present case seems to bear out this presumption.

The conclusions we may draw from the two significant individuals described in the previous pages are: that the development of the female soma of *Thelia* is not influenced by the presence of gonads of the opposite sex; that the changes in parasitized male *Thelia* are not due to the absence of testicular tissue, and that the development of the gonad into ovary or testis is primarily determined by the chromosomal composition of its cells, but the growth of the testis is in part dependent upon the soma which nourishes the germ cells.

8. DISCUSSION

In the arthropods transformation of various male sexual characteristics to corresponding female characteristics has been known and adequately described only in crustaceans infested with parasitic cirripeds. Giard in a series of papers and Smith more recently have identified themselves enduringly with this problem. Smith not only described the external changes of the hosts, but went into an analysis of the effects of the parasites on the hosts' metabolism, using both histological and chemical methods of attacking the problem. He also opened up the field for a discussion as to the manner in which female characteristics are made to appear in the male.

Early in the study of the effects of parasites on sexual characteristics, the term 'castration parasitaire' became associated with this phenomenon, chiefly through the work of Giard. This term leads one immediately to look upon the transformation as being due to castration effected by parasites. This association of castration with changes in sexual characteristics may be attributed to our knowledge of the important influence exerted by the gonads of vertebrates, and especially mammals, upon the development of the soma. Whether effected experimentally or by parasites, castration produces striking results in the higher animals. It is therefore natural, since such parasites as the rhizocephalans or in the case of *Thelia*, *Aphelopus*, generally cause the gonads of the host to undergo reduction or complete obliteration, that we should associate the somatic changes incurred with the loss of the testes or ovaries. Still at the present time there is no evidence in the work on arthropods to support such an inference. It is therefore urged that the term 'castration parasitaire' be set aside in considering the alterations induced by parasites on the sexual characteristics of arthropods. There are several lines of evidence drawn from our knowledge of the crustacea and insects to support this contention.

That the gonads of insects do not produce hormones which shape the development of the secondary sexual characteristics is shown by the numerous cases of successful experimental castration of immature individuals, followed by development

into adults normal in every way—in color, form, and psychic traits. Castration was often followed by the implantation of gonads of the opposite sex and still no effect was induced. Likewise, the injection of gonad extracts into castrated individuals proved ineffective. The most noteworthy of these experiments has been reviewed in some detail in part 2. As intimated, these results are contrary to what is known in vertebrates.

The literature dealing with castration and transplantation of gonads in vertebrates is so voluminous that no attempt will be made here to review the entire subject and only the general principles founded upon these researches will be cited. Several lines of work on vertebrates indicate that the interstitial cells of the gonads secrete important hormones which influence the development of the soma. Castration itself practiced on immature animals causes the retention of juvenal characteristics, and a partial or complete lack of the appearance of morphological and psychic sexual characteristics which normally appear at sexual maturity. The long bones of the body continue to grow and reach a length greater than the average in both sexes; in the male, an unusual amount of fat may be laid on, the external genitalia may be greatly reduced in size, and the male temperament suppressed. However, some characteristics of the sex operated upon may be retained in part, such as the shape of the pelvic bones. Evidence that the interstitial cells and not the growing germ cells themselves play the important part in hormone production is presented by the following phenomena. In cryptorchism, or in testes subjected to ultraviolet rays, active germ cells may be entirely absent while the interstitial cells remain intact. In such animals the secondary sexual characteristics develop normally and it is believed that the activity of the interstitial cells accounts for this. Tandler and Gross ('13) have presented this subject in a most illuminating manner. That hormones play an important part in the early embryonic development of mammals has been shown in the case of two sexed twins of cattle having anastomoses between the circulatory vessels of the two individuals (Lillie, '17; Chapin '17). The more rapidly developing male by its hormones affects the

development of the external genitalia, the oviduct and uterus, and the structure of the gonad of the female twin which becomes a free martin. Not only the interstitial cells of the testis produce hormones, but it is known that the ovary likewise makes important secretions. Steinach ('12) feminized rats by castrating young males and implanting ovaries. These animals later displayed many female characteristics of skeleton and hair pattern; they developed mammary glands and psychic activities of the female, while the external genitalia remained small and undeveloped. In birds Goodale ('16) has conclusively shown that the absence of the ovary allows the male secondary sexual characteristics of the species to be fully developed in the female. Goodale believes that the ovarian hormone acts as an inhibitor, which normally prevents the appearance of those characteristics that we associate with maleness.

Since such intimate association of somatic characteristics with the presence or absence of gonads exists in the vertebrates, it is not surprising that the results on the castration of insects presenting absolute independence of somatic characteristics should be doubted and criticised. Kammerer ('12) would lead us to believe that those who followed in Oudemans' ('98) footsteps and extended the work on the castration of insects, investigators who improved the methods and succeeded in the implantation of gonads of the opposite sex, had interpreted their results blindly, merely accepting the belief in the fixity of the somatic sexual characteristics of insects as propounded by Oudemans, and extending his observations and ideas without question as to their correctness. Certainly, Kammerer's attitude seems rather extraordinary, for the researches of Meisenheimer ('09) and Kopeć ('11, '13, *a*, '13, *b*) seem strictly scientific, well planned, accurate, and convincing. But aside from these experiments, gynandromorphs, such as are occasionally met with in the arthropods, are convincing evidence of the independence of somatic development in this group of animals. One half of the body may be perfect in its male characteristics and the other half female, and within the abdomen there may be present either testes or ovaries or gonads of both sexes. In fact, all possible

internal combinations may be associated with a bisexual soma. Likewise, such an anomalous individual as the *Thelia* nymph shown in figures 52 to 54 is most convincing proof that the testes produce no hormone which influences somatic development. In this individual, a nymph of the fourth instar with soma of a female and gonads of a male, the female secondary sexual characteristics were perfectly formed. Had the same conditions existed in this *Thelia* nymph as exists in developing vertebrates (Lillie, '17), the testes even though immature would have greatly modified the female somatic characteristics. It seems, therefore, most probable that, in the insects and probably in the arthropods in general, the development of the secondary sexual characteristics is independent of the gonads. If we accept this, it follows that the effects of parasitism upon the sexual characteristics cannot be traced directly to the destruction of the gonads.

A second objection to the term 'castration parasitaire' as a descriptive term for the effect of parasites on arthropod hosts is the fact that often the gonads are not wholly destroyed. Smith ('10) showed that if the parasitic barnacle were removed from the host, the germinal epithelium might regenerate and produce germ cells which however in the male might grow into oöcytes, instead of spermatocytes. In bees parasitized by *Stylopidae* the gonads of the male may still be functional, but in all cases the ovaries are described as being very minute. In *Thelia* generally the presence of large *Aphelopus* larvae had as its accompanying condition the entire absence of gonads. Nevertheless, occasionally in males remnants of testes were found, varying from minute clumps of germ cells imbedded in a mass of fatty tissue, to a full-sized testis, such as was shown in figure 50. In parasitized females minute ovaries were at times discovered. The largest oöcytes found in such ovaries were 0.4 mm. in length. That a full-sized testis appearing normal in every way and filled with sperm was found in a parasitized male showing considerable somatic alteration is, I believe, convincing proof that the somatic changes found in parasitized male *Thelia* are not due to the absence of testicular tissue.

Since somatic changes may occur in animals not castrated by the parasites, we must look for these changes as being due to other factors than the lack of gonads. Smith, in his *Studies on the Experimental Analysis of Sex*, always denied the production of hormones by arthropod gonads, and still, in his discussion of the effect of *Stylops* on *Andraena* ('14) he concluded that the reduction of the pollen-gathering apparatus of females and the appearance of male coloration of the face, as described by both Perez ('86) and himself, were accounted for by the reduction of the ovaries through parasitism; just as in the birds the absence of the ovary or the presence of a small non-functional ovary has as its consequence the appearance of the male secondary sexual characteristics. This explanation of stylopization is open to several objections. It omits consideration of the results of experimental castration of female insects. Likewise, in the Hymenoptera, to which order of insects *Andraena* belongs, gynandromorphs have been frequently found (Wheeler, '03): the female portions of the soma appearing perfectly developed, although dissection of several individuals showed the absence of ovarian tissue. Likewise, in social bees, if the lack of development of ovaries would bring about the appearance of male characteristics or the reduction of female characteristics, the workers which are sterile females with small undeveloped ovaries might be expected to have small scopae and present various characteristics of the male. Smith's standpoint, if we are to interpret it from his other papers on sex, might be expressed as follows: the absence of the ovary brings about these changes not because an ovarian hormone has been eliminated, but because there is no ovary present which makes a demand upon the vegetative tissues of the organism. This lack of demand for food material to be stored by the ovary brings about a change of metabolism, and also has as its consequence the somatic alteration already referred to. Whether such a demand is normally made by the gonads of arthropods upon the soma will be considered later.

All evidence points toward the conclusion that the soma of insects is in no way dependent on the gonad in its development. If this be true, we must dismiss in our analysis of the changes in

parasitized Thelia all explanations based upon the experimental evidence gained from operations on vertebrates. As was previously stated, vertebrates and especially mammals castrated as immature individuals often retain juvenal characteristics and continue to grow beyond the normal size, and males may store up a large amount of adipose tissue. Throughout the description of the changes incurred by adult parasitized Thelia, the normal nymphal characteristics have been presented for comparison, and it may safely be stated that in no case were the alterations of males referable to the retention of juvenal characteristics. Only in one case was arrest of development encountered, and that in the genitalia of the fifth instars which contained large *Aphelopus* larvae. These individuals had genitalia resembling those of the fourth instar. It is unfortunate that such individuals are always killed by the emergence of the eruciform larvae, for it would be extremely interesting to know, could they become adults, whether they would possess genitalia similar to those of the fifth instar instead of reduced adult genitalia, such as are met with in all infected adults.

It was seen that parasitized male Thelia grew to a greater size than normal males, but this can hardly be ascribed to continued growth similar to that occurring in castrated mammals. Such an explanation would not account for the fact that parasitized female Thelia are not larger than normal females and parasitized females lack gonads even more frequently than do infected males. Likewise, no increase in size of experimentally castrated insects has ever been reported, and it is quite safe to say that parasitized male Thelia are larger than normal males, not because the testes may be degenerate, but because the parasites exert some positive effect upon the soma which in part develops certain female extragenital secondary sexual characteristics of which greater size is one. This increase in size of parasitized males was noted in the pronotum, wings, head, acrotergites, hind legs, and abdomen. Not merely, therefore, is the region containing the parasites, the abdomen, enlarged, but the most remote portions of the body respond. As far as could be ascertained, this is the first case of this sort found in parasitized

insects. One might offer a simple explanation, that the abdomen increases because the parasites make this a mechanical necessity and this change induces an adaptive increase in other parts of the body: the wings must be larger to carry the increased bulk of the abdomen through the air; the thorax must be larger to contain wing muscles of increased strength. But there are serious obstacles to such an explanation. Very often the parasites do not distend the abdomen of the host at its final molt and still the sclerites are so enlarged that later these larvae which increase tremendously can be accommodated. The influence is, therefore, not by mechanical stimulus. In many cases of parasitism in insects it has been observed that, although the abdomen may be greatly enlarged, the other parts of the body either remain normal in size or are even reduced. Wheeler ('10) cites several cases in which ants with enlarged abdomens containing mematodes possess heads of normal size and wings greatly reduced. Perez ('86) states that in stylopized *Andraenae* the heads of the bees in both sexes are smaller than normal. It is, therefore, rather remarkable that male *Thelia* increase in all parts of the body when infested by *Aphelopus*. We would naturally expect that the drain upon the nutritive material of an immature host would result in a starved undersized adult.

In *Thelia*, although the infected males are larger than normal, still, in this sex as well as among the females, the parasites induce certain changes due to their demands upon the host. The gonads which are not essential to the life of the host are the first tissues to suffer and the material which would go in to the formation of countless numbers of spermatozoa or be used in the growth of ova doubtlessly affords one of the chief sources of nutriment for the parasites. The chitin, too, is often thinner than in normal individuals, this being noted on the pronota of females as well as males. The punctures may be smaller and shallower and the amount of melanic pigment restricted to the depressions of the punctures. Lack of materials demanded by the growing larvae might explain the absence of yellow pigment in the vittae of parasitized males but it would not explain the assumption of the female pigmentation nor would it account for

any of the qualitative changes previously described. Female secondary sexual characteristics are stimulated to development in male Thelia containing *Aphelopus* larvae, and these characteristics are not only those of size, coloration, and pattern, but metabolic characteristics also. The change of male metabolism, I believe, is necessitated by the demand for food by the parasites.

The metabolic differences between male and female tissues are known in many groups of the animal kingdom. These differences are associated primarily with gamete production, the act of fertilization, and the rearing of the young. The female must not only provide material for her somatic needs, but she must often store up a large quantity of food in the formation of ova or furnish it to the growing embryo. The male produces microgametes generally in immense numbers and actively seeks the female, and, with the fertilization of the eggs, often his function is performed. The sexes of Thelia exhibit many differences associated with gamete production. The female develops less rapidly than the male, but the resulting adult is of greater size. Upon reaching the imaginal stage only minute ova are present in the gonads, but the tissues have the capacity for the storage of reserve food, and this storage continues for several weeks, during which time a large amount of yolk and fat is accumulated due to steady feeding and low oxidation of the ingested food. Male Thelia develop more rapidly and appear in July, generally before any females are to be seen. They are already sexually mature at molting and are much more active than females, flying or jumping more easily upon being disturbed. This activity is doubtlessly associated with seeking the females in mating. The males are also less long-lived than the females, disappearing in late summer, whereas the females are still abundant in the fall. The more rapid development of the male, the rapid division of the spermatogonia, the short growth period of the spermatocytes, early sexual maturity, great activity, and earlier death indicate high metabolism and high oxidation. As the season progresses, the gonads of the male become smaller, many cysts of germ cells undergo degeneration, and the stored adipose tissue surrounding the testes decreases in amount.

Thus, as adults, the metabolism of the two sexes stands in contrast: the female is continually storing food material in the formation of ova, the male is using up available material faster than it is being supplied by intake of food. With this difference in metabolism, it is clear that, while the adult female can supply the growing *Aphelopus* larvae which may happen to be present in her abdomen, the male is not so well equipped by nature. The male tissues must change their metabolic level if the host itself is to survive and if the parasites are to obtain sufficient food for their growth. This changed metabolism expresses itself in various ways. As was stated on page 546, parasitized adult males make their appearance in the field along with the females, a week or two later than normal males, indicating that their development has been retarded. They are less active than normal males, sitting immovable on the branches and feeding continuously. They become animals of high storage capacity, as is indicated by the increase in adipose tissue. The portion of the abdomen normally occupied by the testes is filled with a mass of greenish or yellowish fat, often containing remnants of testicular tissue. The parasites themselves are literally imbedded in fat, and a comparison of histological cross-sections of abdomens of parasitized and normal males shows the great increase of adipose tissue in the former. I am greatly indebted to Dr. Oscar Riddle for a quantitative chemical analysis of the alcohol-soluble substances in normal and parasitized *Thelia*. Lots of ten individuals minus pronotum and head were preserved in alcohol for analysis. Two lots of parasitized males and one lot of normal males were analyzed. The parasitized males showed on the average an increase of 47 per cent of lipoids over the normal males. While the samples analyzed were too small and too few in number to be entirely satisfactory, yet they indicate the true state of affairs.

Another change brought about by the parasites is seen in the production of melanin, which is reduced over the entire body of males with the exception of the punctures on the vitta. These are pigmented in parasitized, but not in normal males. This general reduction of melanin may be due to a decrease in the

amount of either the base of melanin, probably tyrosin, or the oxidase, tyrosinase, or possibly to a decrease in both. It has been found that the amount of the ferment present has a great influence on the melanin produced (Kastle, '09), increasing pigment production up to a certain concentration of the tyrosinase, and then inhibiting the reaction. It is also known that even weak acids prevent melanic pigment production, and the parasites of *Thelia* probably bring about a condition of acidosis in the host, which has as its most important result fatty infiltration of tissues, and which also may bring about inhibition of melanin formation.

Recognizing that the metabolic level of parasitized male *Thelia* has been altered from the normal, are we to refer the changed morphological somatic characteristics as being due directly to this change? The answer to this question rests entirely on our conception of the origin and meaning of sex itself. If we believe that the underlying difference between the sexes is one of metabolism and not one of gamete production, then high metabolism has as its consequence maleness and sperm production, while low metabolism has femaleness and egg production as a consequence. The opposite view is that primarily the male is a sperm producer, the female an egg producer. The cells of an individual, somatic as well as germinal, are of one sex, either male or female. The metabolic level of the organism is merely one of the many expressions of sex, but a very important one in a consideration of gamete production and the nourishment of the offspring. This difference in metabolic pitch certainly is more important than other secondary sexual differences, as size, pattern, coloration, or psychic characters, and probably preceded these in the phylogenetic development of sex. It has probably become more divergent in the two sexes as the gametes have been modified in evolution, as active seeking of the female by the male and internal fecundation has become established in higher forms and the development of the embryo has become dependent upon the female. Just as the form of the sperm or egg must be molded by the genes in the cells of the individual which produces them, likewise, it is not improb-

able that the metabolic level also depends on genetic factors probably present in the chromatin.

The excellent researches of Smith on sacculinized crabs indicated that the metabolic level of infected male crabs was lowered. Smith would ascribe the appearance of female secondary sexual characteristics in these crabs as being referable to the underlying causes of the changed metabolism. Lowered metabolism evinced itself in various ways. It was first detected by the presence of lipochromes characteristic of the blood of the female (Smith, '11, '13, and Robson, '11). Then it was shown that the percentage of fat in the blood of infected males increased, approaching that characteristic of females producing ova. Histological sections of the liver (Robson, '11) showed a great increase of fat globules in the cells of parasitized males, bringing about a condition similar to that of normal females. Smith ('13) made quantitative analyses of the livers of normal and infected individuals and showed that fat production was stimulated in parasitized male crabs and that glycogen production was depressed bringing about a metabolism characteristic of the female. Smith contended that the *Sacculina* roots act as does a normal ovary. They make a demand on the tissues of the host and in the male alter the substances carried by the blood and body fluid. This demand has two results, it brings about the increase of fat molecules and it stimulates the production of certain female secondary sexual characteristics. In Smith's theoretical considerations of the problem he explained the demand made by the *Sacculina* roots and the response of the tissues upon the Ehrlich theory of immunity reactions. The *Sacculina* roots in parasitized males acting upon protein molecules in the crab's blood stimulate the production of fat links, which travel through the blood to the host's liver, where each receives a fat molecule which it transports back through the blood to the *Sacculina* fat chains. Here the fat molecule is given up and the fat link again travels toward the liver. The constant increase of fat links stimulates an increase of fat molecules in the liver of the host. Smith clearly states that not the increased fat itself in the blood stimulates the appearance of the female secondary sexual char-

acteristics, but the deep-seated changes or underlying causes which involve increase of fat stimulate the appearance of the secondary sexual characteristics. Just how the Sacculina roots or normal ovary bring about the production of changes in the blood is not gone into, but certainly the whole differs little from the hormone theory which Smith himself opposed vigorously. In Smith's explanation, the Sacculina roots or ovary act upon molecules in the blood and the changed blood acts upon the somatic tissues. In the hormone theory, the gonad produces substances within its own cells, and these substances transported by the blood which acts merely as a carrier affect the somatic cells to which they are brought. The most questionable feature of Smith's theoretical considerations is the demand of the ovary upon the soma, followed by a response on the part of the somatic cells. In the arthropods we are wholly without evidence that there is any demand coming from the ovary which can alter the metabolism of the vegetative cells or stimulate the production of certain somatic characteristics.

Such an individual as that described by Wenke ('06), whose work was reviewed in part 2, offers difficulties to a belief in ovarian influence exerting an effect upon the developing soma. The individual described was a perfect lateral gynandromorph with the male somatic characteristics perfectly developed in one half, although the only gonad present was one well-developed ovary containing eggs almost mature. Such combinations of male and female soma are not altogether uncommon in the insects and higher crustacea and indicate that in the arthropods the genes in the cells producing the somatic structures are not influenced either directly or indirectly by the gonads.

Another line of convincing evidence on the question of the independence of somatic tissues in insects is that brought forward by the works of Steche ('12) and Geyer ('13), as previously reviewed on pages 536 to 537. These investigators proved that the haemolymph of insects was unlike in the two sexes, that sometimes color differences were evident in phytophagous species and that there were always protein differences demonstrable by precipitation tests. Castration failed to alter the characteristic

color or composition of the haemolymph. Likewise, the implantation of testes into castrated female caterpillars or the implantation of ovaries into castrated males failed to alter the characteristics of the haemolymph in either of the sexes operated upon. The conclusion of both authors was that the somatic cells which produced the haemolymph, referring principally to the cells of the digestive tube, were either male or female, and by their activity the haemolymph was also male or female in its characteristics. The function of these digestive cells was clearly shown to be independent of the ovary or testis. Surely, if the ovary were capable of creating a demand upon the soma, we would expect that an ovary transplanted into a castrated male would so affect the cells of the digestive tube that the necessary materials for provisioning the ova with yolk materials and pigment would be supplied. But as a matter of fact, implanted ovaries in male somas fail to get their necessary supplies and they fail also to influence those somatic cells which, instead of being nutritive in function, have for their mission the production of the more permanent structures which include many of the secondary sexual characteristics. In the work of Kopeć ('11) we see a strengthening of the conclusions of Geyer and Steche upholding the physiological difference between the somatic cells of the two sexes. It is well known that ovaries transplanted into castrated males never attain normal size, being generally a third or a fifth as long and containing but a fourth or fifth the normal number of ova which are much undersized. A further observation of Kopeć ('11) is interesting, namely, that in *Gastropacha quercifolia* such ova are yellow instead of green and have fewer and smaller yolk granules than eggs of normal females. This latter condition, I believe, may be traced to the fact that the cells which provided the haemolymph were male instead of female and thus the failure to supply the green pigment and necessary yolk materials. Kopeć ('11) and Meisenheimer ('09) would refer the smallness of the implanted ovaries to lack of space for development in the smaller male abdomen, but if the ovary were capable of making a demand as postulated by Smith we would expect that those male larvae in which

ovaries had been implanted would as adults possess abdomens capable of accommodating the mature ovary. In sacculinized male crabs and in male *Thelia* parasitized by *Aphelopus* the abdomen is enlarged, but there is little evidence to support the idea that the enlargement is called forth by an influence similar to that supposedly exerted by an ovary. The physiological activity of the somatic cells, although correlated with gamete production, does not, in insects, seem to be governed by the gonads, but is probably to be referred to the genetic constitution of the cells upon which the gametes themselves may be partially dependent for their normal development. This is illustrated by the anomalous *Thelia* nymph described in part 7. This individual, a nymph of fourth instar, had perfect female soma and contained two testes which were undersized for this stage and contained many degenerating cysts. If we compare the normal development of the gonads in the two sexes, we see that the testes develop rapidly and are full sized at the final molt, but the ovaries progress very slowly and are smaller than the testes during the entire nymphal life. In the adult female they grow to relatively enormous size, but in the fourth instar they are considerably smaller than the testes. Thus, in the anomalous nymph, I believe, we must ascribe the smallness of the testes to the fact that they were provided with only as much nutriment as a female soma normally provides to its contained ovaries. The degenerating cysts indicate that cell division proceeded faster than materials for growth were supplied. Here surely the testes failed to influence the soma to change its normal metabolism or to produce any male characteristics. If, then, the developing soma of arthropods is entirely independent of any influence emanating from the gonads, we cannot explain the modifications produced by parasites on the assumption that the parasites act as do the gonads of one sex or the other.

The sexual characteristics of insects must, I believe, depend entirely upon the chromatic makeup of the cells composing the individual. Of all the insects, no groups show a more universal visible chromatic difference between the cells of the two sexes than do the Hemiptera and Homoptera. It is not my

purpose to review here the many papers on hemipteran and homopteran chromosomes, so it will suffice to say that, in spite of the many combinations found, the presence of one x-chromosome in the cells of the male and two x-chromosomes in the cells of the female is the essential difference. It is true that each x-chromosome may be represented by a group of separate chromatic elements and that the x-chromosome may be accompanied in the cells of the male by a y-chromosome, but these are rather subordinate details. We also know that this sexual difference extends not only to the cells of the gonads, but to the somatic cells as well (Morrill, '10, Hoy, '16). The writer has definitely demonstrated that this also holds true for *Thelia*. The growing gonapophyses of the fifth nymphal instar were slipped out of their chitinous coverings and mounted in acetic carmine. Many clear and handsome metaphase plates were studied and drawn with the aid of a camera lucida. The female somatic cells showed regularly twenty-two chromosomes including two large x-chromosomes, whereas the male cells exhibited twenty-one chromosomes, of which the largest one was the x-chromosome. Two of these cells are shown in figure 51, *b* and *i*. Thus the sex of the soma and gonads of insects is determined in the zygote, and there is at no time an indifferent sex gland which may be molded into testis or ovary by the soma in which it develops. This idea of the indifferent gonad was put forth by Doncaster ('14), who, after reviewing the work on the arthropods describing the physiological differences between males and females, states that perhaps the physiological differences are the primary sexual differences and that the 'primitive gonad' develops into an ovary on one hand or into a testis on the other in consequence of this. The peculiar nymph described on pages 594 to 598 of this paper illustrates nicely that the chromosomes determine the character of both the soma and the germ plasm. This individual with the soma of a female had male gonads and a cytological examination showed that these gonads had the male complement of chromosomes. In some way the primitive germ cells which formed the gonads failed to receive two x-chromosomes, and so testes developed instead of ovaries. That the soma was

female did not prevent the development of testes, nor did the presence of testes in any way interfere with the development of the female somatic characteristics. In insects the chromatin of the germ cells represents a mechanism for shaping the gametes, and this mechanism is extremely difficult to upset, and cannot be interfered with by metabolic changes in the soma.

This is nicely demonstrated in parasitized male Thelia. Many careful microscopic examinations of some forty testes and remnants of testes from parasitized males have failed to reveal any cells taking on the characteristics of oöcytes. That the metabolism of these males was altered by the growing *Aphelopus* larvae cannot be in the least questioned. They were no longer animals of high oxidizing powers, but stored fat in large quantities and in many other ways approached female metabolism. Still spermatocytes failed to grow beyond their normal size, and often, when conditions were not too adverse, divided into spermatids which differentiated into mature spermatozoa. Frequently, fatty infiltration caused the spermatocytes to degenerate, and one might infer that conditions in parasitized individuals always made continued growth of the germ cells impossible. This, however, cannot be the case, for in parasitized females oöcytes measuring 390μ by 46μ have been found, and these had grown in the adult females from cells no larger than 56μ by 36μ , which is the maximum size of oöcytes at the final molt. Therefore, storage is possible in the germ cells of parasitized adults. But spermatocytes are not induced by changed conditions to approach in character the germ cells of the female, although the spermatogonia may have been subjected to the changed environment for many generations. That the gamete-forming mechanism is rather stable is shown also by Goldschmidt's intersexual moths ('17 b). He states that the last organ to be changed to that of the opposite sex is the gonad. Likewise in vertebrates, where the development of the sexual characteristics is largely dependent on hormones, the gametocytes are not easily influenced. Thus in the gonads of the freemartins, although the general structure of the ovary is profoundly changed by the male hormones, still the germ cells fail

to develop into gametes of the opposite sex (Chapin, '17). This stability exhibited by the germ cells is probably due to some fundamental difference early established in the evolution of sex between spermatogonia and oögonia or between spermatocytes and oöcytes.

Were it permissible to speculate upon the origin of the sexual differences, the writer would consider the production of two sorts of gametes as the first and fundamental step. It would be assumed that originally either in the protozoans or lowest metazoa the individuals were isogametic and that zygotes were formed by the union of two similar, rather large, and not extremely active gametes, as still exist among many green algae and certain protozoa. If by a mutation one individual produced many small, highly motile gametes which sought the larger less active ones in conjugation, the number of zygotes might be increased and the species thereby benefited. This original mutation which established microgamete production would require a more rapid cell division and a shorter growth period in gametogenesis. The gene bringing this about would be represented in the constitution of all the cells of the individual, somatic as well as germinal, and might influence in many ways the form and physiology of the whole individual. Recently Morgan ('17), in speaking of the manifold effects of each gene, citing an example from *Drosophila*, says that, "whatever it is in the germ plasm that produces white eyes, it also produces these other modifications as well and modifies not only such 'superficial' things as color, but also such 'fundamental' things as productivity and viability." A gene in one chromosome may influence or inhibit the genes located in other chromosomes and change in many ways the characteristics or constitution of the mutant. But even in the origin of heterogametic forms should the soma of the individual producing the microgametes (the male) remain similar in form to the macrogamete producer (the female), as it does in many marine forms, including even annelids and echinoderms, still there would remain that genetic difference in all the cells of the individual, and any future mutation would arise either in the presence or the absence of the gene affecting the fundamental difference between the sexes.

It must be assumed that the gametes themselves have undergone an evolution and that many genetic changes have occurred to modify and specialize the form of the mature germ cell. The multitudinous forms and the intricate apparatus which spermatozoa exhibit lend credence to this idea. Likewise, the ovum must have been changed from its original state. One of the first processes, one which has now become universal in animal ova, must have been the production of cells of unequal size in maturation. The polar cells represent abortive ova, whereas the one functional ovum receives the nutritive supply originally intended for four cells of equal size. Thus fewer and larger ova would result from this change. In many animals the period of growth was prolonged so that larger ova resulted, finally culminating in the immense egg cells of birds. Undoubtedly mutations affecting the storage power of the somatic cells as well as the germ cells have aided in this specialization and caused the female to diverge in its physiological constitution from the male. This difference is still demonstrable between the male and female somas of mammals. Although the ovum no longer stores yolk, still the body tissues must provide food for the growing embryo. An economy is perhaps hereby effected in that fertilization must first be insured before prolonged storage of food materials can take place.

With the specialization of the gametes changes in the accessory tissues of the gonads, in their ducts and glands, must have become established. These tissues minister to the needs of the gametes and serve to carry them to the exterior. In land animals and also in some aquatic animals copulation and internal fertilization made the development of external genital apparatus essential and the establishment of instinct of sex necessary. Last of all, we are to look upon the extragenital sexual characteristics, including all forms of ornamentation, as coming into existence.

According to the views expressed above, sex has evolved through a series of genetic changes accompanying the evolution of the various groups of the animal kingdom. It is not assumed to have sprung up independently whenever a difference in the

rate of metabolism of the germ cells of individuals in the various groups appeared (Riddle, '17). Metabolic differences are demonstrable and measurable between the sexes of highly specialized forms which store yolk in large ova or provide nutriment to the zygote. This metabolic level is assumed to be an expression of sex rather than a causal factor.

Genetic changes are most probably to be sought in changes in the chromatin. In the evolution of sex, genes located in various chromosomes have undoubtedly played a part. In nematodes, insects, spiders, and some mammals visible chromatic differences have led to the belief that the unpaired element of the male cells, the x-chromosome, is intimately connected with sexual differentiation. Let us inquire what is known about the x-chromosome and the location of other genes affecting principally sexual characteristics. In spermatocytes the x-chromosome would seem entirely different from ordinary chromosomes. It does not form a typical leptotene thread, and even if a y-chromosome is present it fails to form a double syndetic thread with this element. Should a fairly long growth period follow syngamy and the autosomes become very indistinct, the x-chromosome remains a compact deeply staining mass. This is true for *Thelia* and is shown in figure 51, *c* and *d*. Still, the x-chromosome is not an inert mass of chromatic material differing in character from that of the autosomes. When paired as in the oöcytes, they behave exactly like any of the other chromosomes. They form leptotene threads which conjugate. In *Drosophila* (Morgan and Bridges, '16) linkage and crossing over in the paired x-chromosomes has been shown to occur just as in the autosomes. By investigations of linkage many genes which bring about sex-linked characteristics have been located in the x-chromosome and these genes seem to have a definite linear arrangement. Furthermore, Bridges' ('16) remarkable observations on 'non-disjunction' have definitely demonstrated that one x-chromosome is absolutely necessary in the formation of the male and that two and only two are essential in the production of a female. From the behavior of the x-chromosome in spermatogenesis one might infer that this element were in-

active, an unessential in the male, and that males might be produced lacking this element, yet no zygote in the 'non-disjunction' experiments formed by the union of an ovum minus an x-chromosome and a sperm also without this chromosome developed. Likewise, ova containing two x-chromosomes never developed if fertilized by spermatozoa containing an x-element. Thus females with three x-chromosomes are not formed. These same experiments of Bridges also demonstrated that the y-chromosome is without influence in sex production for xxy females and xyy males are in no way different from normal individuals except in the types of gametes produced. No genes have been located in the y-chromosome, yet from its behavior in spermatogenesis it seems to have some affinity for the x-chromosome, and it is not improbable that the y-chromosome represents an altered x-chromosome rendered inactive through some change in its makeup. Its affinity for the x-chromosome is shown in the formation of an unequal xy tetrad in the first spermatocyte division, as in the Coleoptera, or an unequal xy diad in the second spermatocyte, as in the Heteroptera. In *Enchenopa binotata* (Kornhauser, '14) these two chromosomes unite end to end in syndesis and form a tetrad composed of two elements similar in size. Thus, I believe, we are to look upon the y-chromosome as having originally been the partner and homologue of the x-chromosome, but that a change, perhaps the loss of a single gene, made it inactive as a carrier of genes. Inactive and therefore unimportant, it might undergo many chance variations or losses which might culminate in the final disappearance of the y-element, a condition not at all uncommon in the insects. Bridges ('17) has recently shown that a chromosome may become deficient as a bearer of genes. A race of flies was produced in which the x-chromosome was abnormal in that a particular 'measurable section of genes' was either inactivated or lost. This experiment further demonstrates that 'deficient' x-chromosomes produce normal sex ratios, and he concludes that the determiner of sex is not the 'x-as-a whole,' but that in some definite part or parts of the x there are specific sex-differentiators.

If the production of an animal with but one active x-chromosome had as its consequence, perhaps through further mutations in the one-x-individual, the formation of a microgamete-producing individual, the beginning of sexual differentiation would be established. The inactivation of the x-chromosome or the production of the y-chromosome may merely have brought about a less stable condition in the cells of the individual possessing the inactive x-element, and offered a basis for further mutations leading to the differentiation of two types of individuals, one producing microgametes and the other macrogametes. The further divergence of the sexes in the form of the gametes and soma would be partially dependent upon this primary difference in stability or constitution, for new genes would necessarily arise and be expressed either in cells with one or with two functional x-chromosomes. That there is something vital in the x-chromosome upon which development depends was shown nicely by Bridges, who proved that zygotes without an x-chromosome or with more than two x-chromosomes failed to develop. It is therefore not unlikely that cells having two sets of x-genes should be somewhat differently constituted than those possessing but a single set. The male often shows a much greater tendency toward variation than does the female, and this most probably is referable to a greater conservativeness in the composition of the female cells. In the family of the Membracidae, with which the author is fairly familiar, the variability of the males is much greater than that of the females. In *Thelia* the form of the pronotal horn and the extent of the vitta, as illustrated in figure 7, page 556, exhibit far less constancy than in the female. Fowler ('03) has described and figured many types of the membracid *Umbonia orozimba* which in the male show many types of variations in color, form, and size. Some greatly resemble the females, but the series extends to forms so unlike the female that they were originally placed in another genus, *Physoplia*, by Amyot and Serville ('43). As stated on page 589, the membracids are characterized by conservativeness of the genital appendages. Lately the author has studied the genitalia of both sexes of forms from the various subfamilies

of the Membracidae, and in more detail the available genera of the tribe Telamonini, to which *Thelia* belongs. The outstanding result is that, whereas the female genitalia show a remarkable conservativeness to a general type, even in the most widely separated forms, the male genitalia are not nearly so stable. All the genital appendages of the male, the oedagus, the claspers, and ventral valves, show a diversity of form not approached by the female gonapophyses. Even the abdominal sclerites of the male become involved and form accessory apparatus to the genital appendages. The greater inconstancy of the male is, I believe, due to the fact that new genes may find expression in individuals possessing a single x-chromosome in all their cells which could not find expression in the presence of two x-chromosomes.

Factors to be important in heredity must arise in the germ cells. They must arise therefore either in the spermatogonia and oogonia or in spermatocytes and oocytes. New genes must have their origin in cells which are male, or cells which are female. It is conceivable that a mutation might arise in a germ cell of a male which could not arise in a female germ cell owing to greater stability and conservativeness of the latter, due to its chromatic constitution. Likewise, the expression of any new gene must often be entirely dependent upon those genes already established in the heredity of the individual. Those genes already fixed in the evolution of the family, genus, or species form, as it were, the internal environment for the new gene. The production of characteristics in the development of an individual are believed to come through a series of changes in the protoplasmic mass governed and controlled by the genes located in the chromatin. Each step must be dependent upon those steps which preceded, and thus the most recent characteristics must be dependent on the changes produced in the cells by the genes older phylogenetically.

The egg of an insect even before fertilization becomes highly differentiated. This subject has recently been presented by Hegner ('17). The regions of the cytoplasm become specialized, polarity and bilaterality are established. We look upon the force which determines and brings about this organization

as emanating from the nucleus of the growing oöcyte. After fertilization the division of the cleavage nucleus gives rise to a number of nuclei, and these possessing complete diploid sets of chromosomes migrate to various parts of the cytoplasm which become more and more specialized as development proceeds. This specialization becomes rigid, as shown by the experiments on centrifuging insect eggs, destroying portions of developing eggs (Hegner, '17). In the latter class of experiments, the uninjured portion continues to develop and forms only that part of the embryo which would be formed by it in a normal egg. However, we do not believe that even in insects the cells of the embryo act as independent units, although this condition becomes almost realized in the formation of the adult. In the embryo the cells coöperate to form various organs, and there must be some vital intercellular connection. As development proceeds in an insect egg the internal mechanism of the individual cells seems to be of the greatest importance in shaping the most highly differentiated tissues of the adult. Crampton ('99) showed this nicely in his lepidopteran experiments. Hypodermis grafted onto a transforming pupa of another species or of opposite sex developed as it would have in the individual from which it came, as shown by the pigmentation and cuticular out-growths of the ingrafted portions.

Must we not also look upon the development of sex in an individual as a continuous series beginning with the differentiation of the gonads, proceeding in the formation of the important accessory sexual organs and culminating in the expression of various specific secondary sexual characteristics? Those sexual differences old phylogenetically are developed early in ontogeny and each of the more recent genes affecting sexual characteristics must be dependent upon the entire series of changes which preceded. Every cell of the individual possessing a complete set of chromosomes probably has a double set of genes representing the total hereditary basis of the species. There is a single exception to this, namely, that in the male the genes located in the x-chromosome are present only once. We know from the various lines of evidence presented on pages 539 to

544 that each sex possesses the genes for the secondary sexual characteristics of the opposite sex, but that normally one set finds expression in the male and the other set in the female. Thus they differ from sex-linked characteristics which may be present in either males or females, although this distinction is sometimes not recognized. The 'exclusively male characteristics' studied by Foot and Strobell ('15, '17 a, '17 b) belong to the secondary sexual characteristics which in the cases best known have their genes located in the autosomes. The above-named investigators have shown in their *Euschistus* crosses that the gene or genes controlling the length of the intromittant organ may be transmitted from father to son, and that, since the son receives no x-chromosome from the male pronucleus, this gene or group of genes must be located in the autosomes. It does not seem improbable or impossible that the expression of a particular set of genes for the secondary sexual characteristics, even though located in the autosomes, should be dependent upon some controlling gene or genes in the x-chromosome. With the knowledge of the multiple effects of a single gene, and the presence of modifying and inhibiting factors, more and more evidence is being accumulated to show that a particular factor in one chromosome may influence the expression of genes in other chromosomes. The writer would contend that the important and vital genes of the x-chromosomes, not those forming the sex-linked characteristics, but those which must be present either once or twice for development to take place at all, create, if present once, an environment for a series of changes leading to the expression one by one of the male characteristics, and, if present twice, lead to the development of the female characteristics. If we consider for a moment the ontogenetic series in Thelia, we find that in the embryo one may see the differentiation of the gonads into ovaries or testes. The arrangement of the gonia, the form and method of attachment of the tubules, the structure of the gonaducts, and the position of the genital apertures early distinguish the males from the females. The external genital appendages, developing most probably from the primitive abdominal limb buds of the eighth to tenth somites,

early become distinguishable as male or female, and this difference becomes more and more apparent as development proceeds. In the fourth and fifth nymphal instars the somatic cells as a whole show physiological differences in that the females grow more slowly, but grow to a greater size. Last of all, with the final molt and sexual maturity, comes the expression of a host of secondary sexual characteristics. Pigmentation, size, form, and detail of the sclerites show various sexual differences and the gonapophyses exhibit their specific characteristics. The difference in metabolic pitch now becomes very evident as the female stores yolk material, while the more active male uses up his stored energy. Behavior differences connected primarily with the act of reproduction also appear in the imagos.

In the insects we know of no hormones produced in the gonads either early or late in ontogeny, which, circulating in the blood, constitute a factor in the internal environment of the somatic cells stimulating certain genes to find expression and suppressing others. In vertebrates such hormones certainly exist, and are generally believed to be produced in the interstitial tissue of the testis or ovary. They are different in the two sexes, as shown by their effects upon developing somas. These hormones must be produced through the influence of certain genes which are active in the interstitial cells. That the hormone may be modified by new genes has been demonstrated by Morgan's ('17) experiments on hen-feathered Seabright cocks. In these birds the testis produces a hormone which prevents the development of the normal male plumage and causes a large proportion of sterility, according to Goodale ('16). That the sex-hormone-forming genes of the male find their expression in the presence of one x-chromosome while those of the female depend on two x-chromosomes seems not unlikely. The hormones from the gonads and probably in some cases from other endocrine glands circulate in the blood and form a very important step in sexual development, creating at times a necessary factor for the expression of those genes which follow in their activity in the series of developmental changes. But while no definite hormone forms an important step in the sexual development of insects, still each gene

as it comes into activity must cause a change in the protoplasmic mass which it influences and, since the cells are not independent but in physiological continuity, this constitutes a definite step also. Since each gene is represented in every cell of the individual, its coming into particular activity in one part of the organism might be accompanied by minor changes due to this same gene in other parts of the organism. In both arthropods and vertebrates I would look upon the sexual development of the individual as a continuous series, each step depending on the steps preceding. The presence of one x-chromosome would form the basis for the inauguration of the male series, beginning in the formation of a sperm-forming gonad and ending with the production of specific secondary sexual characteristics. Likewise, the presence of two x-chromosomes in the zygote would start the female series of developmental changes.

Returning finally to the effect of *Aphelopus* upon *Thelia bimaculata*, will the foregoing considerations help us any in understanding the changes suffered especially by the male in its secondary sexual characteristics? It was shown that the germ cells of the male were not changed toward those of the female type, nor were the male gonapophyses altered so as to be similar to those of the female, although they were reduced in size. These reduced external genitalia, although still retaining the general form common to the membracids, lost their specific characteristics. In the fifth instar it was noted that parasitized individuals of both sexes showed a retardation in the development of the gonapophyses. The striking changes brought about were in those characteristics of the male which first appear at the final molt and belong to the category of the extragenital characteristics. Especially noteworthy was the loss of the male coloration and the assumption of the female pigment and pattern. Likewise, the female arrangement of the spines on the abdominal sclerites and increase in size of all parts of the body were plainly observed in parasitized males. It must also be remembered that parasitized males formed a complete series from those but slightly altered to those with extreme change. Those but slightly changed, if taken shortly after the final molt,

showed the presence of only small parasites, and it seemed reasonable to believe that the degree of change was largely due to the size of the parasitic larvae during the fifth instar. Not by their size merely, but by their greater physiological effect, would the presence of the large parasites greatly alter the internal conditions. The degree of change in the host's form would be directly referable to the degree of internal alteration effected by the parasites. It might be expressed as a quantitative reaction depending on the concentration of the metabolic products of the *Aphelopus* larvae. The sexual characteristics of *Thelia* which appeared early in ontogeny would be subjected to the products of the parasites' metabolism during several molts, although the concentration of these products must be rather less in the earlier instars. Yet in spite of this the older characteristics we found were not reversed. Spermatogonia were surely subjected to conditions where the growth of oöcytes would be possible and still only spermatocytes developed. The external genitalia were greatly reduced in size, but still retained the general form found in the Membracidae. Their reduction in size is probably due to the fact that the step from the genitalia of the fifth nymphal instar to the adult form is a big one, the size difference being rather remarkable in the two stages; and, just while this growth is taking place in parasitized individuals, the *Aphelopus* larvae must exert a greater influence than they did in the previous instar or instars when smaller in size. It may also be true that it is difficult to reverse the development of an organ old ontogenetically and well established in the group after it has once got under way. There also seems to be good reason to believe that the more recent the characteristic and the more specific it is the more it will be altered. The details of sexual differentiation often represent various specializations of phylogenetically older differences. The specific characteristics of the gonapophyses come under this category, and thus we find that the oedagus and ovipositor of *Thelia bimaculata* are altered in regard to these details when parasites are present. Giard ('89) showed that the elaborate oedagus of *Typhlocyba hippocastani* lost its specific characteristics when parasitized by *Aphelopus*,

so that this organ could no longer be used in distinguishing hippocastani from certain other species.

The color reversal in *Thelia bimaculata* was very complete in many parasitized males. If we examine various examples of the tribe Telamonini, to which *Thelia* belongs, we find that the coloration of the male is not often strikingly different from that of the female. In *Thelia bimaculata* we have an exceptional and extreme case of dimorphism in coloration. Thus in *Thelia uhleri* the nearest relative both sexes are of a reddish-brown color. Microscopical examination shows that a faintly outlined lateral area on the pronotum corresponding to the vitta of *bimaculata* exists and that the melanic pigment of the pronotum is chiefly restricted to the punctures. No sexual difference either in the pigment or in the pattern on the pronotum or face could be found. The coloration certainly resembles that found in the female of *bimaculata* much more than the male, and I believe we are to look upon *Thelia uhleri* as representing a more ancestral type. There are several reasons for this. The gonapophyses are quite similar in form to those of other genera of the Telamonini, as indicated by the bulbous oedagus and pointed ovipositor. Also the arrangement of the spines on the abdominal terga shows a greater similarity in the patterns of the sexes than was found in *Thelia bimaculata*. The rows in the male are not so straight and close together and the characteristic network arrangement of the female may also be found over parts of the male terga. Thus I believe that the coloration and arrangement of the abdominal spines in the male of *Thelia bimaculata* represent recent changes and that the female characteristics represent the more ancient and less modified condition. These recent sexual characteristics of the male must have arisen through genetic changes modifying characteristics once common to both sexes. The genes for these characteristics of the male find their expression in the final ontogenetic step in the presence of one x-chromosome, and they probably depend upon all the changes in development which preceded them. Is it not likely that the internal upset caused by the parasites interferes with the normal expression of these male genes?

Let us briefly review the possibilities of internal alteration which might affect the cells. The growing larvae depend entirely upon the host for their respiratory and nutritive needs. Oxygen must be extracted from the haemolymph or produced by the reduction of carbohydrates in the haemolymph. Carbon-dioxide must be poured back into the haemolymph by the parasites. It is not unlikely that full-grown larvae distending the abdomen of the host interfere with its respiratory movements. Should an insufficient supply of oxygen be furnished the cells of the host, a condition of acidosis would result in the tissues. This would bring about the accumulation of droplets of fat in the cells and lead to the degeneration of many cells. Microscopic examination shows much fatty infiltration and the accumulation of adipose tissue around the *Aphelopus* larvae. This tissue probably forms one of the chief sources of energy for the development of the parasites. But not only energy is needed, proteins must be supplied as well by the haemolymph of the host. In growing the parasites must give rise to nitrogenous waste materials. The premeagathic larvae, which can alter an adult considerably if present before the final molt, probably excrete these waste materials directly into the haemolymph of the host. In the meagathic larvae at least part of the katabolic products are deposited as insoluble crystals in the digestive tract. There is a possibility that nitrogenous excretions from the larvae might be toxic. Parasitic worms have long been known to give rise to toxic excretions (Fürth, '03). These toxins might act indirectly upon the developing cells. In *Thelia*, as in all homopterans probably, there exists a tissue well organized and of considerable size, arranged segmentally in the abdomen, and known as the pseudovittelus. This tissue, in which symbiotic fungi develop, is thought to be very important in the metabolism of the animal. In parasitized *Thelia* the pseudovittelus is much reduced, although I have seen no indications of fatty degeneration in its cells. This reduction of the pseudovittelus might also alter the composition of the haemolymph. With the possibility of so many alterations in the haemolymph which bathes the cells and furnished them with food and oxygen, a

complete solution of the problem as to the one important change seems rather remote. We would maintain that the changes were not effected through the production of some sex hormone and that the parasites do not act like an ovary to stimulate the development of the female secondary sexual characteristics in males. It seems rather that the altered haemolymph failed to provide the necessary conditions for the expression of the characteristics most recent in the evolution of *Thelia bimaculata*, namely, the specific characteristics of the gonapophyses and the male extragenital sexual characteristics. These new characteristics may have come into the species by the origin of genes modifying established characteristics, and the failure of these genes to come into activity would produce an organism retaining ancestral characteristics. The male and female individuals would then be more similar in color, size, and in other details of their integument. A slight change in the environment would permit a partial expression of the newest genes, and thus intermediates were found in males containing small larvae.

9. SUMMARY

1. *Thelia bimaculata* is parasitized by the dryinid *Aphelopus theliae*. An egg of the parasite may be deposited in an immature *Thelia* of any of the five nymphal instars. The *Aphelopus ovum* undergoes polyembryonic development and gives rise to about fifty larvae which reach their maximum development either in the fifth nymphal instar of the host or in its adult stage. The *Aphelopus* larvae escaping from their host, thereby killing it, drop to the ground, burrow in, pupate, and become mature the following summer.

2. *Thelia* parasitized by *Aphelopus* show many alterations. Most interesting are the changes wrought in males which reach the adult form. These assume either partially or completely many sexual characteristics of the female, much as do sacculinized crabs. The degree of change depends upon the size of the parasites during the fifth nymphal instar of the host. If the *Aphelopus ovum* is deposited early in the nymphal life of the

host, the parasitic larvae will be large and the assumption of the female characteristics pronounced; if deposited late, the alterations will be less marked.

3. Of the changes in parasitized males none is more striking than the assumption of the pigmentation of the female. The character of the pigment and its distribution on the pronotum and head may duplicate exactly that of the female.

4. Parasitized males increase in size, approaching but not reaching the size characteristic for female *Thelia*. Measurements show this increase in the pronota, wings, heads, legs, acrotergites, and abdomens. Thus all regions of the body are influenced and the amount of increase is correlated with the degree of alteration of the pigmentation, those with complete female coloration being largest.

5. Parasitized female *Thelia* show no assumption of male pigmentation, nor do they change in size.

6. Minute spines on the abdominal sclerites of parasitized males take on the arrangements characteristic of the female. The shape, pigmentation, and texture of the abdominal sclerites of parasitized males become female in character and various sclerites of the terminal somites associated with the genital appendages show considerable change toward the opposite sex.

7. Parasitized individuals of both sexes sometimes show a weakened cuticula and a reduction of the melanic pigment.

8. None of the changes described are due to a retention of nymphal characteristics.

9. The genital appendages of parasitized males are not changed to those of the opposite sex. They are reduced in size and lose their specific characteristics, but retain the general form found in male Membracidae. Likewise, the gonapophyses of infected females show a similar decrease in size and a loss of specific characteristics, but retain the general form found in female membracids.

10. The above (9) may be partly explained by a history of the gonapophyses. The genital appendages are laid down early in ontogeny and become clearly sexually differentiated in young nymphs. Thus the sexes may be easily ascertained by an

external examination of the nymphal gonapophyses of the third instar and by an examination of sections much earlier than this. These genital appendages are ancient ontogenetically and phylogenetically as well, belonging to a type primitive for the group of the Homoptera. The small parasites of the younger instars are not capable of changing the growing sexually differentiated nymphal gonapophyses to those of the opposite sex. They may retard the development of these appendages in both sexes, so that fifth instars with full-grown parasites present gonapophyses quite similar to those found in fourth instars.

11. The parasites generally cause the degeneration of the gonads and bring about an accumulation of fat in the abdomen of the host. Testes and ovaries in various stages of disappearance were studied. Never were cells similar to oöcytes found in any testes, but normal spermatogenesis proceeded as far as possible under adverse conditions. Likewise, oöcytes retained their characteristic features and even grew for a time in some parasitized adult females.

12. One parasitized male was found which, although considerably changed toward the female condition, contained a full-sized normal testis with many spermatozoa. Another individual, a fourth instar, showed perfect female soma, but contained male gonads composed of cells with the characteristic male complex of chromosomes. The first individual indicates that the changes wrought by the parasites are not due directly to the destruction of the gonads, while the second individual lends support to the idea that the soma of arthropods is independent of the gonads in its development. Likewise, there is no evidence that the demand for food made by the parasites on the soma of male hosts stimulates the development of female secondary sexual characteristics. Such a demand has been assumed by other investigators to emanate normally from the ovaries, calling forth the development of the female characteristics.

13. It is not thought probable that the lowering of the metabolic level in parasitized males could account for the changes described.

14. Male membracids show a greater degree of variation than do females. Males seem to have a less stable constitution and have departed from generalized types farther than have females. This difference in constitution probably rests on the chromatic makeup of the two sexes—the xx condition being more stable than the xy or xo condition. The male of *Thelia bimaculata* shows extreme departures from the female in many characteristics of form, size, color, and pattern. The male extragenital sexual characteristics and the specific characteristics of the gonapophyses first appear in the final molt. The extragenital sexual characteristics may be due to genes which in many cases modify characteristics once common to both sexes, but now exhibited in a primitive condition only in the more stable female. The metabolism of the parasites, altering the constitution of the host's haemolymph which bathes the developing cells, may offer an environment unsuitable for these recent genes to find their expression and leave the male individual in a more primitive state exhibiting characteristics now found in the female of the species.

Evanston, Illinois,
May, 1918

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La musculatura facial de los japoneses.

El presente trabajo trata de los músculos faciales y se basa en la disección de quince japoneses, tres chinos y cinco europeos, comparándose los resultados obtenidos con los de otras razas. La musculatura de los mongoles está generalmente menos diferenciada y es mas primitiva que la de los europeos, aunque en diversos casos sucede lo contrario. El autor dá detalles sobre cada uno de los músculos de la cara.

Translation by José F. Nonidez
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THE FACIAL MUSCULATURE OF THE JAPANESE

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FIVE TEXT FIGURES AND THREE PLATES

Since Chudzinski and Giacomini made their contribution on the muscular system of the negro, many works have been published, especially on the head musculature of the intra-European races (Forster, Birkner, Fischer, Eggeling, Fetzner, Loth, H. Virchow). These authors have uniformly proved that racial differences are related to facial muscles. The following study is intended to be a small contribution to the solution of the problem.

The material at my disposal consists of five Europeans (male), three Chinese (male), and fifteen Japanese (ten male and five female). All are adult. The Japanese cadavers came mostly from hospitals, others from prisons (among these two that had been hanged). I arranged them in the following order under the respective abbreviations: for Europeans, EI to V; for Chinese, CI to CIII; and for Japanese, JI to JXV (and likewise the males JI to JX, females JXI to JXV).

The faces have been dissected on both sides or on a single side (usually the right); the condition of nourishment of Europeans and Chinese is good; that of the Japanese is as follows:

- JI, body no. 3778, age 51, emaciated.
- JII, body no. 3768, age 18, good.
- JIII, body no. 3048, age 44, good.
- JIV, body no. 3750, age 18, moderate.
- JV, body no. 3780, age 21, moderate.
- JVI, body no. 3765, age 51, emaciated.
- JVII, body no. 3762, age 30, moderate.

- JVIII, body no. 3145, age 20, good.
- JIX, body no. 3756, age 45, good.
- JX, body no. 3786, age 21, emaciated.
- JXI, body no. 3772, age 20, moderate.
- JXII, body no. 3781, age 32, good.
- JXIII, body no. 3770, age 61, emaciated.
- JXIV, body no. 3659, age 21, moderate.
- JXV, body no. 3729, age 28, good.

The Japanese heads were separated from the bodies and injected with a carbol-glycerin-alcohol solution. The European and Chinese heads were first salted and frozen, were then exposed for a long time to formol fumes, and have been preserved for many years in alcohol. In order to preserve the color of the muscles, several heads were immersed in sodium chloride or sodium nitrate solution. In the preparation for study a preparation needle was also used; furthermore, I have studied the more delicate fibers in alcohol or water as Eisler has done.

I have made measurements of the muscles of the European and Chinese, mostly on both sides of the face; but with the Japanese I have always made the measurements on one side only. Attention may be especially called to the fact that the differences of muscularization have been confirmed, mainly by direct comparison of the preparation with one another by the unaided eye. The proportions based on measurements are not sufficiently trustworthy.

Furthermore, it is to be noted that the majority of the European authors in their studies of the facial muscles of extra-European races do not show how they have compared these with the Europeans. One cannot discover whether the authors have prepared the European faces specially for this or not, what kind of preparations they were, nor how many heads of Europeans were used for the comparison. In the comparison of the (evidently not numerous) relationships of facial muscles, it is absolutely necessary that they may be worked out by a juxtaposition of the dissections.

At this place it is fitting that I express my heartiest thanks to Professor Doctor Adachi for his manifold suggestions and the liberal gift of the excellent material for unconditional use.

My study divides itself into two portions:

1. The individual facial muscle.
2. The facial musculature as a whole.

1 THE INDIVIDUAL FACIAL MUSCLES

*Platysma myoides*¹ (figs. 1 to 3)

In all of my material this muscle shows the greatest variety in the muscle configuration of the face, hence I have not as yet been able to discover any apparent difference between the Mongolian (Japanese and Chinese) and European types. Loth has rightly observed that it is erroneous to suppose that in a race in which one expects a primitive musculature of the face, the platysma should always be well developed.

The negro has, at times, well-developed muscles (Chudzinski, Giacomini, Turner, Eggeling, Loth), at other times they are very poorly developed (Giacomini, Loth). Among the Papuans, as far as dissections have shown, the muscle is said to be well developed (Forster, Fischer, Steffens, and Körner-Eckstein). On the other hand, Fetzner has not expressly mentioned the primitive character of the platysma in the study of seventeen Hottentot heads.

In general, in the Japanese and Chinese, the platysma fibers arising at the edge of the jaw are extensive and form a closed muscle plate, a fact which Birkner has observed in three Chinese heads. I have made a similar observation also on three European preparations (EIII, EIV, EV).

The fibers diminishing toward the corner of the mouth and directed toward the lower lip (pars labialis) run more transversely or upward and forward in the Mongolians and are not plainly separated from the bundles ascending in the orbitotem-

¹ For practical purposes the platysma is visible only in the cheek and neck portions of the separated head.

poral direction (*pars aberrans ascendans*). The latter section radiates at various elevations near the region of the cheek.

I have found in five Japanese and two Chinese² (figs. 1, 3, 4, 6, and 7) well-developed fibers which cross a line drawn from the corner of the mouth to the outer auditory opening, but I have not found these in European heads. The platysma which reaches this line has been observed in four Japanese and three Europeans.³ I have observed in six Japanese (fig. 5), a Chinese (fig. 2), and two Europeans (fig. 6)⁴ a lesser extension of the muscle, which usually exists as the *pars labialis* and does not reach a line drawn from the corner of the mouth to the auditory organ.

I have arranged these three developmental stages after Loth, as follows:

	JAPANESE (KUDO)	CHINESE (KUDO)	NEGRO (LOTH)	EUROPEAN (KUDO)
Strong.....	5	4	18	0
Medium.....	4	0	6	4
Weak.....	6	2	2	3
Number of half faces.....	15	6	26	7

The radiating form of the cheek division of the platysma is subject to many variations. These have been arranged by Bluntschli-Loth in the following series of types:

I. Usually transverse course of the upper platysma-fibers (fig. 8).

Ia. Weakening of type I.

The above two types belong to a primitive group.

II. Acquisition of an ascending direction of the fibers with loss of the transverse. *Pars aberrans ascendans* is well developed (figs. 1, 4, 6, and 7).

IIa. *Pars aberrans* is lost or only weakly developed.

III. Strengthening of *pars labialis* after loss of *pars aberrans* (fig. 5).

IIIa. Weak development of the platysma, the fibers of which hardly extend over the edge of the mandible.

² JII, JIV, JVIII, JXI, JXV, and CII, CIII.

³ JVII, JIX, JX, JXV, and EIII, EIV, EV.

⁴ JI, JIII, JIV, JV, JX, JXIII, CI, and EI, EII.

However these types grade into one another and there are other possibilities of platysmal relationship. The divisions are therefore more or less arbitrary.

	JAPANESE (KUDO)	CHINESE (KUDO)	EUROPEAN		NEGRO (LOTH)
			(Birkner ¹)	(Kudo)	
Type I.....	0	0	0	0	5
Type Ia.....	2	0	0	0	5
Type II.....	6	4	2	0	12
Type IIa.....	5	2	1	4	2
Type III.....	1	0	0	2	12
Type IIIa.....	1	0	0	1	0
Number of half faces.....	15	6	3	7	36
		9			

¹ After Birkner's figures.

Loth contends that the primitive relationships, which recall the primates, are relatively abundant in the negro. He calls special attention to the fact that types IIa, and IIIa are present in large numbers in Europeans. Likewise it is not exceptional that four of the seven half faces of my Europeans belong to type IIa.

In the Mongolian types II and IIa are common. It may be noted here that the pars aberrans ascendens directed upwards is often bent forward over the cheek region like a bow and in two Japanese it divides the platysma-risorius (see also M. risorius).

In its distribution the platysma is variously related to the adjacent muscles. In the negro the interweaving of the muscle with the M. triangularis, zygomaticus, quadratus labii sup., orbicularis oculi, etc., has been observed many times. The muscle, in the Mongolian, is always covered with the triangularis and usually with the fibers of the risorius (figs. 1, 2, 3, and 7). In two Japanese and two Chinese (fig. 3) the platysma reaches the zygomaticus, where the fiber ends of the former cover those of the latter. Furthermore, in a Chinese (CII, fig. 7), the platysma reaches to the splitting up of the bundles of the orbicularis oculi; in a Japanese (JVI), beyond that point.

The cervical region of the platysma has been observed in three Japanese (figs. 1, 2), a Chinese (fig. 7), and a European. In these Chinese (fig. 7) the cervical bundles are not directly connected with the cheek region of the platysma. In this (CII), as fig. 7 shows, the neck fibers of the platysma first run ventral from the front, then, forming an open angle above the ear, arise from the anterior region dorsad, and finally interweave with the radiating bundles of the orbicularis oculi.

Under the chin the fibers of either side cross over to the opposite side in three Chinese heads. I found this crossing to be the rule in the Japanese cadavers.

Fig. 1. Male Japanese VI, fifty-one years old. Platysma well developed; pars aberrans ascendens rises above the line from the angle of the mouth to the auditory meatus. The risorius is spread over the platysma in several strands; intermediate strands occur between this and the triangularis. The zygomaticus is penetrated at its insertion by the caninus and split into a superficial and a deeper layer of the quadratus labii superioris; the three heads together form a nearly continuous whole. The caput zygomaticus, at its origin, is close to the radiating bundles of the orbicularis oculi anterior. The bundles which turn downward in the middle of the course of the caput zygomaticus, pass below the zygomaticus; those which extend from the orbicularis oculi are well developed at the lateral (especially the superior lateral) and median inferior portion of the muscle. The M. occipitalis is moderately broad; its fibers are transverse. A proper M. transversus nuchae lies under the posterior bundles of the occipitalis at the same level as the lowest belly of the auricularis posterior; other fibers, which may be the remains of the platysma bundle run close beneath, obliquely downward and forward. The larger auricularis posterior is divided into three parts and, in an almost fleshy condition, reaches the ear cartilage.

Fig. 2. Chinese I. The platysma is poorly developed and runs almost transversely in front; the pars aberrans is lacking. The triangularis is covered at its origin by risorius and platysma fibers. The risorius is distinct from the triangularis. The zygomaticus is divided into two portions which pass into each other; the upper part extends close to the caput zygomaticus of the quadratus labii superioris and at its insertion, becomes lost in the caninus; the lower portion extends downward toward the triangularis. The caput zygomaticus of the quadratus labii superioris connects with the caput infraorbitale. The orbicularis oculi forms a well-closed ring, although the lateral bundles enter the caput zygomaticus. The lateral short strands of the frontalis are extended toward the temple. The lower bundle of origin of this muscle joins the upper lateral part of the orbicularis ring. The M. occipitalis spreads out fan-like and reaches the anterior fibers of the auricularis superior. The auricularis superior and anterior is fibered vertically; the auricularis posterior is plainly separated into two long bellies. The transversus nuchae is lacking. The cervical part of the platysma is directed obliquely over the insertion of the sternocleidomastoid.

Figure 1

Fig. 3. Chinese III. On the whole, the facial musculature has quite firm fibers. The platysma forms a single mass; the pars aberrans, divided like a V, extends beyond the line from the angle of the mouth and the auditory meatus; the posterior portion reaches the lateral marginal bundle of the orbicularis oculi, and the anterior passes, beneath the orbicularis, beyond the zygomaticus major and minor. The M. triangularis is broad at the edge of the jaw. The risorius shows considerable development; the zygomaticus is penetrated at its insertion by the insertion bundles of the risorius and triangularis. The three heads of the quadratus labii superior, in part covered by the orbicularis ring, form a continuous muscle. The coarsely bundled orbicularis oculi is radiately extended at its upper lateral part. Noteworthy are the large parallel fibers which have lost their connection with the auricularis and course over the temple in the same direction as the latter. Finally, oblique fibers are directed toward the frontalis; farther forward a transversely fibered extends toward the front under the insertion of the frontalis. All such thin-fibered sheets may belong to the auriculo-frontalis.



Figure 2



Figure 3

M. mandibuli-marginalis *Blundschli* (*M. menti accessorius*
Kelchi) (*fig. 6*)

This muscle is found in Europeans only as an infrequent variation (Kelchi, Wood, Henle, Testut, Ruge, Seydel, Blundschli). Ruge has observed the muscle as a variety arising from the platysma, while Seydel considers it the remnant of a sphincter colli superficialis.

I have encountered the muscle twice in the Japanese (in JVIII and JXII) in fifteen half faces. Thus it appears to be no rarity in the Japanese. In the latter (*fig. 6*) many isolated fibers extend from the prolongation of the triangularis toward the ear. They lie superficially on the cheek portion of the platysma and cross that muscle. In the former two separate muscle strands radiate from the margin of the jaw bone upward in the form of a weak bow, concave on the anterior face. In a Chinese head Birkner has seen similar compact muscle strands directed toward the ear.

M. triangularis (*figs. 1 to 7*)

In Mongolians this muscle is mostly fan-shaped behind, below, and in front. As far as can be judged in my material, it is better developed in the Chinese than in Europeans. Likewise Birkner found this muscle well developed in three Chinese heads; the same is the case in the negro (Flower and Murie, Hamy, Hartmann, Chudzinski, Popowsky, Eggeling and Loth) and in the Hottentots (Fetzer).

In the Japanese and Chinese (*figs. 3 and 7*) the triangularis is commonly associated with the risorius; in Europeans, on the other hand, the muscle is usually isolated (four out of five heads). I have seen the well-known variations of the insertion ends of the fibers (Eisler), namely, the transition of bundles into the *M. caninus*, in five Japanese, one Chinese and three European heads; the transition into *M. zygomaticus* in one Chinese and seven Japanese; the transition into *M. orbicularis oris sup.* in nine Japanese, two Chinese, and two European heads. I have never met with a tendinous interruption of the muscle at the angle of



Figure 4

Fig. 4. Female Japanese XII, thirty-two years old. Platysma well developed; pars ascendens arcuate forward and extends to the zygomatic. The zygomaticus is easily distinguished from the caput zygomaticus of the quadratus labii superioris. The insertion of the zygomatic is separated by the caninus into a superficial and a deeper layer. The risorius consists of only two arcuate muscle bands and lies over the insertion of the triangularis. The three heads of the quadratus labii superioris are comparatively easily distinguished; the caput zygomaticus at its origin radiates toward the temple. The diverging marginal bundles of the orbicularis oculi, like the upper lateral part, are especially large in the lower medial portion; the latter pass over the infra-orbital head of the quadratus labii super. to the caput zygomaticus. The pars transversus of the M. nasalis is present. The occipitalis is weakly developed and short-fibered. The M. auricularis posterior has two bellies. The long cervical portion of the platysma passes forward in a light curve over the insertion of the sternocleidomastoid under the ear.

the mouth. The fibers of the triangularis, already mentioned, which run into the orbicularis bundle have frequently been found before in the negro (Chudzinski, Popowsky, Loth). The insertion of the muscle in Mongolians is usually at the upper median part of the risorius bundle (figs. 1 to 4 and 6). I have not observed the division of the triangularis into several large portions (Macalister) in the Mongolians.

In order to show the extent of the muscles, the following measurements (in millimeters) have been inserted, although they are merely of general interest.

1. The breadth of the muscle at the lip commissure, according to Chudzinski, is 15 (12 to 19) in the negro on the average; 10 (6 to 15) in the Japanese (fifteen individuals); on the right side 6, 10, 12, on the left side 9, 12 in the Chinese (three individuals); on the right side 7, 11, 14, on the left side 7, 8, 9, 10 in Europeans (five individuals) according to Kudo, according to Chudzinski 11 on the average.

2. The breadth of the muscle at the place where the risorius diverges, is 20 (10 to 33) in the Japanese; on the right side 15, 25, 20, on the left side 10, 27 in the Chinese; on the right side 14, 20, 20, on the left side 13, 19, 15 in Europeans; 11 to 15 in the Hereros (Eggeling).

3. The breadth of the muscle in the region of radiation is 38 (29 to 55) according to Chudzinski, 37 (31 to 43) according to Eggeling and Loth in the negro; 45 (30 to 60) in the Japanese; on the right side 40, 65, 45, on the left side 35, 70 in the Chinese; on the right side 31, 35, 44, on the left side 30, 25, 40, 35 in the European, and according to Chudzinski an average of 38.

Fig. 5. Male Japanese III, forty-four years old. The pars aberrans ascendens of the platysma is lacking. The risorius consists of two arcuate bundles. Zygomatic separated at its insertion into a superficial and a deeper layer by the caninus. The caput infraorbitale is separated in the region of origin from the other two heads. The lateral marginal bundles, and especially the upper, are developed. The M. frontalis extends farther up, its lateral fibers are short and arcuate toward the ear. The auricularis is spread out like a fan; its posterior fibers are transverse; the anterior are interrupted for a distance. The occipitalis is strongly developed; its hinder fibers are more vertical, the anterior are inclined forward, interlaced with the auricularis superior, and finally reach the conch in a fleshy condition. The auricularis posterior is divided into two portions, the lower of which undergoes a tendinous interruption.



Figure 5

4. The divergence of the median muscle edge from the middle of the chin amounts to 14 (9 to 19) in the negro (Chudzinski); 13 (5 to 20) in the Japanese; on the right side 6, 6, 12, on the left side 12, 14, 15, 9 in the Chinese; on the right side 6, 10, 9, on the left side 12, 14, 15, 9 in the European, and according to Chudzinski an average of 12.

M. risorius (figs. 1 to 8)

Opposite the risorius of Santorini, which arises from the triangularis, Ruge has distinguished a platysma-risorius, in which Forster and Loth support him. This is said to arise by a continuous separation from the arcuate platysma bundles extending over the cheek toward the angle of the mouth. Such a distinction, however, appears to have no significance, since there are cases where actual confirmation is difficult, even impossible. Such a recognition would be especially difficult in the Mongolian, because here the platysma bundle is directed very generally toward the corner of the mouth, covering the risorius. Still the typical platysma-risorius is occasionally present, as I have seen it in at least three cases in Japanese preparations. These three cases are grouped according to the arrangement of Blundschli, one (JIV) of type VI, 2 (JV and JX) of type IV.

In the Mongolians the *M. risorius* is recognized, as is the triangularis, by its stronger development. My material allows the following arrangement. Observations on fifteen half faces of Japanese:

1. Risorius lacking (fig. 8): twice in Japanese, once in European.
2. Risorius is weakly developed and entirely isolated from the triangularis (fig. 4): four times in Japanese, two times in Europeans.

In JIII, JIV, and EI, EIII, platysma bundles are inserted between *M. risorius* and triangularis and have a convergent course, together with the former, toward the corner of the mouth. They may function possibly as a risorius.

3. Risorius is spread out radially in the region of its origin; several intermediate bundles are present between risorius and triangularis (figs. 1 and 6): 6 times in Japanese, 2 times in Europeans, once in Chinese.

4. Risorius spreads out into a fan pattern, forming together with the triangularis a closed muscle plate (figs. 3 and 7); 3 times in Japanese, 2 times in Chinese, none in Europeans.

Birkner is also struck by the more powerful development of the risorius. According to the account of Chudzowski, this muscle in the negro is also more markedly developed; yet, as Loth remarks, this author has not seen the true risorius. Egge-ling has found the risorius better developed but once in five Hereros.

The M. risorius is more rarely lacking in the Mongolian than in the black race. I missed the muscle only twice in fifteen Japanese heads, not once in my three and Bickner's three Chinese heads. In the negro Loth enumerated fifteen cases out of thirty-five half faces, in which the muscle was entirely lacking (thus about 43 per cent). In the Hottentots Fetzer usually found the muscle lacking; that is, he found the muscle on both sides in only four heads out of fifteen Hottentots and on one side in only two more. Unfortunately, we have no account which tells us how often the muscle is lacking in Europeans.

Blundschli divides the muscle into six types. The compiling of this classification is rendered difficult, however, by various transition forms. The approximate frequency of the types is brought together in the following table:

	NEGRO (LOTH)	JAPANESE (KUDO)	CHINESE (KUDO)	EUROPEAN (KUDO)
Type I.....	15	2	0	1
Type II.....	5	2	0	0
Type III.....	10	1	1	1
Type IV.....	5	3	2	1
			1	
Type V.....	1	4	1	2
Type VI.....	0	3	1	0
Number of half faces.....	36	15	6	5

M. transversus menti

With regard to the frequency of this muscle, according to accounts of Thiele, Schmidt, and LeDouble, fifty-six cases (60 per cent) in ninety bodies have been found with the muscle present (Eisler). But the number is too high according to Eisler, because it encloses radiating bundles of the platysma and triangularis. The muscle occurs eight times out of twenty-one heads in the negro, according to the computation of Loth. Fetzner failed to find the muscle only once in seventeen Hottentot heads, twice it was only rudimentary. In the Japanese and Chinese the muscle was present without exception; but twice it was only vestigial. Birkner found the muscle readily demonstrable in the Chinese head. It is evident, then, that the muscle is more generally present in the Mongolians and Hottentots than in the Europeans and negro.

The relationship of the transversus to the triangularis and platysma is variable. In the Japanese the muscle is at times derived entirely from the more anterior triangularis fibers, which run in an arcuate manner along the submental region; more frequently (six Japanese heads) the triangularis fibers pass over in part into the transversus. Eggeling has observed a similar condition twice in the negro. The muscle is often entirely isolated, or occasionally inserted at both ends into the edge of the under jaw through the platysma fibers. This muscle has been observed twice in the negro (Eggeling and Loth) without a connection with the triangularis.

The development of the muscle is computed by its breadth, which on the average amounts to: 7 mm. in the negro (Eggeling and Loth); 5 mm. in the Japanese; 3, 4, 5, 4, 2 mm. in the European, and 3, 5, 3 mm. in the Chinese (Kudo).

M. quadratus labii superior (figs. 1 to 8)

All authors state that this muscle is less frequently divided into three portions among colored races than in Europeans (figs. 6 and 8). Fetzner, on the other hand, notes no peculiarities among Hottentots.

In the Mongolians the differentiation of the muscle parts is very slight (figs. 1, 3, 4, 6, and 7). Birkner has observed an apparently united quadratus in a Chinese. In the negro the three distinct portions appeared in only three out of twenty-six individuals. I have found this muscle divided into three parts three times in the Japanese. As a rule, among Japanese the muscle likewise is fused into an entire muscle plate, so that the superficial layer, caput angulare and caput zygomaticus, and especially near the origin, the caput infraorbitale, covers it; if the former is more strongly developed, then the latter is entirely covered by it (in JII, JXIV). In the following lines the individual muscle portions are considered:

The caput zygomaticum (*M. zygomaticus minor*) is well developed and is never lacking in the Mongolians. In the negro (Chudzinski, Loth) the caput was absent in two out of forty-eight half faces (7.7 per cent), while, on the other hand, in Europeans (LeDouble) it was lacking twenty-two times in 100 individuals (22 per cent). The separation of the caput from the *M. orbicularis oculi* is usually difficult, since the radiating bundles of the latter spread out over the surface of the so-called caput; only in the case of three Japanese was the head of the muscle perfectly distinct.

The caput infraorbitale (figs. 1, 4, 5, 6, and 8) is strong in the Mongolians, coarse-fibered, and usually covers the caput zygomaticum (figs. 3 and 6). The caput in the superficial layer in the case of two Chinese is strengthened by means of the orbicularis bundle (fig. 7); but I have never observed a caput divided into two distinct portions in the Japanese. The breadth at the point of origin is as follows:

Japanese (Kudo) ca. 16 (10 to 26) mm.

Europeans (Kudo) right 12, 26; left 12, 22, 21, 16 mm.

Europeans (Chudzinski) 22 mm.

Chinese (Kudo) right 23; left 25 mm.

Negro (Chudzinski) 32 (22 to 39) mm.

The caput infraorbitale is difficult to isolate from the other two capita in the case of the Mongolians (figs. 1, 6, and 7). The well-separated cases are divided into the following ratio:

Japanese (Kudo) two times in fifteen half faces (13.3 per cent).

Chinese (Birkner and Kudo) none in six half faces.

European (Kudo) three times in five half faces.

Negro (Loth) four times in forty-seven half faces (8.5 per cent).

The small muscle which is figured in atlases or in the text-books has been observed by me only once in the Mongolians.

The caput angulare (figs. 1, 4, 5, 6 and 8) may have its origin above the ligamentum papebrale mediale. Its connection with the adjacent muscles is apparently intimate; the union with the frontalis has often been observed. The caput is so intimately connected with the orbicularis oculi that it not only unites with it, but also receives superficial bundles from it.

M. orbicularis oris (figs. 1, 4, 5, 6, and 8)

Birkner has observed a well developed muscle in the Chinese. In the negro the muscle is well developed, in connection with the thick lips; according to Eggeling, even curved, with its free ends respectively somewhat outward and upward in the upper lip, downward in the lower lip.

In the Japanese and Chinese a stronger development as contrasted with the Europeans has not been demonstrated. The muscle appears here as a plate with nearly parallel fibers which, as in Europeans, has an anterior position. In the negro the orbicularis is more fully developed in the under lip than in the upper (Giacomini). I have not been able to establish a clear distinction of development between the upper and the lower lip.

M. quadratus labii inf. (figs. 1, 3, 4, 5, 6, and 8).

In the preparation of the superficial layer this muscle shows no noteworthy differences between the Japanese and the Europeans.

M. zygomaticus

In the Japanese and Chinese this muscle forms a relatively powerful strand and often grows together with adjacent muscles

(figs. 6 to 8). As to the development of the muscle, its breadth⁵ at different muscle levels is arranged as follows:

Breadth at origin:

Japanese (Kudo) 6.4 (5 to 10) mm.

Chinese (Kudo) right 9, 8, 10, left 8, 9, 5 mm.

European (Kudo) right 7, 7; left 7, 10, 10, 5, 5 mm.

European (Chudzinski) average 8 mm.

Negro (Chudzinski) 10.5 (8 to 18) mm.

Breadth in the middle:

Japanese (Kudo) 7.7 (6 to 14) mm.

Chinese (Kudo) right 8, 12, left 11, 9 mm.

European (Kudo) right 9, 10; left 8, 9, 11, 5, 5 mm.

European (Chudzinski) average 7 mm.

Negro (Chudzinski) 10.4 (6 to 14) mm.

Breadth at lip insertion:

Japanese (Kudo) 10.5 (6 to 20) mm.

Chinese (Kudo) right 10, 10, 12; left 11, 9, 6, mm.

European (Kudo) right 18, 14; left 11, 9, 6 mm.

European (Chudzinski) average 25.3 mm.

Negro (Chudzinski) 26 (13 to 32) mm.

This muscle is never lacking in the Mongolians which have been investigated, and is very seldom absent from the Europeans (Otto, Macalister). The division into two (Macalister), three or four portions (Chudzinski, Popowsky) has not been observed in the Japanese and Chinese; but the eccentric orbicularis bundles often merge with this muscle (JV, JVI, JXI, and CII) (figs. 1, 6, and 7), just as Birkner has observed in a Chinese head. In a Japanese head (JVII) the orbicularis bundles entering the muscle turn upward and mesad into the orbicularis ring. In a Chinese and in European heads the muscle is entirely separate from orbicularis fibers (figs. 2 and 8).

The muscle is often combined (in JIX, CII, EII) with the platysma bundle; it is also covered by it. The direction of the fibers is more crosswise or transverse. Chudzinski has measured the space between the head of the muscle and the anterior end

⁵ Measurement is the breadth of the deeper head portion of the muscle where it is covered with the orbicularis bundles.

of the porus acusticus externus. The data secured from this are as follows:

Japanese (Kudo) 50.9 (44 to 66) mm.

Chinese (Kudo) right 48, 40, 50; left 50, 47, 55 mm.

European (Kudo) right 50, 51; left 53, 55, 51, 54 mm.

European (Chudzinski) average 47 mm.

Negro (Chudzinski) 40.3 (33 to 46) mm.

The relation of the zygomaticus in the region of insertion affords great interest. A short distance from its insertion into the lip it is penetrated by the caninus, so that at its end it is split into a superficial and a basal layer (figs. 1, 4, 5, and 7). Such a condition was found three times in the Japanese, only once in the Chinese and Europeans. An insertion, with the superficial layer the stronger, has been found in three Japanese and one Chinese; one with a weaker superficial layer in three Japanese. The superficial layer may be lacking; the muscle end crowds its way likewise deeply toward the buccalis (in a Japanese, a Chinese, and a European, fig. 8). The absence of the deeper portion has been established in three Japanese and one European heads. However, the muscle is always penetrated more or less by the caninus.

A well-isolated zygomaticus is very rare in the Japanese, just as in the Chinese. The following table concerning this may be instructive:

JAPANESE (KUDO)	NEGRO (LOTH)	EUROPEAN (KUDO)	CHINESE (KUDO)
15 half faces 4 (26.7 per cent)	45 individuals 11 (23 per cent)	5 half faces 1	3 half faces 0

Loth believes that the fusion of the muscle with the contiguous structures takes place only exceptionally in Europeans.

M. orbicularis oculi (figs. 1 to 6 and 8)

This muscle consistently shows a moderately strong development in the Mongolians. It forms a powerful broad ring around the eye. In stating the strength of the muscle, up till

now, its breadth from the edge of the eyelid has been taken as a criterion by authors. The following tables give the result of such measurement:

1. Distance of the middle of the upper lid margin from the upper edge of the muscle (with the eye closed):

Negro (Chudzinski, Popowsky, Eggeling, Loth), 28.2 mm.

Japanese (Kudo), 27.2 mm.

Chinese (Kudo), right, 30 mm; left, 24, 25 mm.

European (Kudo), left, 22, 30, 29, 30, 19 mm.

European (Chudzinski), 16 mm.

2. Distance of the middle of the lower lid margin from the lower edge of the muscle (with the eye closed):

Negro (Chudzinski), 25.5 mm.

Japanese (Kudo), 29.6 mm.

Chinese (Kudo), right, 30, 37, 35; left, 30, 32, 35 mm.

European (Kudo), right, 28; left, 29, 23, 25, 37, 28 mm.

European (Chudzinski), 26 mm.

3. Distance of the corner of the eye from the outer edge of the muscle (which can be computed quite exactly, because the contour of the muscle through the eccentric orbicularis bundles or the growth with contiguous muscles) is very little modified.

Negro (Chudzinski), 26.1 mm.

Japanese (Kudo), 31.1 mm.

Chinese (Kudo), right, 30, 30, 30; left, 31, 32, 35 mm.

European (Kudo), right, 28, 33; left, 29, 14, 27, 23 mm.

European (Chudzinski) 22 mm.

From this table it is apparent that the muscle in the Mongolian is broader in the lateral portion; the upper orbicularis portion is smaller than the under; in the negro the relationship is reversed.

According to Chudzinski, the muscle is more powerfully developed in the negro than in the European; likewise in the Hot-tentots. This is apparently the case also in the Mongolian. In the primates the muscle element situated over the edge of the orbit is generally weakly developed (Ruge). I have directly computed the distance of the outer edge of the pars orbitalis from the edge of the orbit; the result obtained is about the same as the measurement at the edge of the lid as given above.

The scattering bundles at the margin of the orbicularis radiate in very different ways in the Mongolian; but strongly developed bundles are relatively rare at the margin of the eyelid. For comparison with the tables of H. Virchow and Loth I separate the bundles into the following groups:

1. Upper lateral marginal bundle. A = the one directed dorsad (mesad). B = the one directed ventrad (lateral).

JAPANESE (KUDO)	CHINESE (KUDO)	EUROPEAN (KUDO)	NEGRO (LOTH)	HOTTENTOT (FETZER)
14 half faces 13 (92.9%) $\begin{cases} \text{A. 13} \\ \text{B. 0} \end{cases}$	4 half faces $3 \begin{cases} \text{A. 3} \\ \text{B. 0} \end{cases}$	5 half faces $2 \begin{cases} \text{A. 2} \\ \text{B. 0} \end{cases}$	38 half faces 20 (52.5%)	17 individuals 14 (82.4%)

2. Lower lateral marginal bundle. A = the one directed dorsad (laterad). B = the one directed ventrad (mesad).

JAPANESE (KUDO)	CHINESE (KUDO)	EUROPEAN (KUDO)	NEGRO (LOTH)	HOTTENTOT (FETZER)
14 half faces 10 (71.4%) $\begin{cases} \text{A. 0} \\ \text{B. 10} \end{cases}$	4 half faces $4 \begin{cases} \text{A. 1} \\ \text{B. 3} \end{cases}$	5 half faces $2 \begin{cases} \text{A. 1} \\ \text{B. 1} \end{cases}$	38 half faces 33 (86.9%)	17 individuals 10 (58.8%)

3. Lower median marginal bundle. A = the one directed dorsad (laterad). B = the one directed ventrad (laterad).

JAPANESE (KUDO)	CHINESE (KUDO)	EUROPEAN (KUDO)	NEGRO (LOTH)	HOTTENTOT (FETZER)
14 half faces 6 (42.9%) $\begin{cases} \text{A. 1} \\ \text{B. 5} \end{cases}$	4 half faces $0 \begin{cases} \text{A. 0} \\ \text{B. 0} \end{cases}$	5 half faces $2 \begin{cases} \text{A. 1} \\ \text{B. 1} \end{cases}$	38 half faces 29 (76.3%)	17 individuals 15 (88.2%)

4. Upper median marginal bundle. A = the one directed dorsad (laterad). B = the one directed ventrad (mesad).

JAPANESE (KUDO)	CHINESE (KUDO)	EUROPEAN (KUDO)	NEGRO (LOTH)	HOTTENTOT (FETZER)
14 half faces 4 (28.6%) $\begin{cases} \text{A. 4} \\ \text{B. 0} \end{cases}$	4 half faces $1 \begin{cases} \text{A. 1} \\ \text{B. 0} \end{cases}$	5 half faces $2 \begin{cases} \text{A. 1} \\ \text{B. 1} \end{cases}$	38 half faces 6 (15.8%)	17 individuals $0 \begin{cases} \text{A. 0} \\ \text{B. 0} \end{cases}$

This shows that the outermost bundles at the lower medial margin, in Japanese and Chinese, are, as a whole, more strongly developed and often consist of larger fibers than in Europeans. At their origins, the bundles, together with the depressor superciliai, often extend over the ligamentum palpebrale mediale; also, united with the caput angulare laterally below, they extend farther than the fasciculi deflexi toward the M. zygomaticus major (JVII, fig. 7) or minor (JVIII). According to Fetzner, the lower median bundle is usually present in Hottentots; this appears to be the case not infrequently in Europeans.

The adjacent orbicularis, well distinct from the contiguous muscles in Europeans (Loth), is only distinguished with difficulty in Mongolians, as also with the negro (Loth, compare fig. 8 and figs. 6 and 7) especially on the upper and lower margins the boundary of the muscle is not distinct. The muscle often unites with the quadratus labii superius and zygomaticus to a compound muscle (fig. 7).

M. depressor superciliai

This is always found as a triangular muscle above the ligamentum palpebrale mediale in Japanese and Chinese; no differences have been observed between Mongols and Europeans. Loth has observed it considerably developed in a negro.

M. corrugator superciliai

This muscle, which comparative anatomy regards (Ruge) as an offshoot of the bundles of the orbicularis oculi occurring above the cleft of the lids, is frequently, both in Mongolians and Europeans (Macalister), not separated from the orbicularis oculi. Similar relations have been shown by Popowsky and Loth for the negro. According to Eisler, this muscle is readily distinguished from the frontalis by its coarse bundle formation. In the Mongolian the muscle, together with the depressor, is generally fused with the frontalis, as in the negro (Chudzinski). It is seldom lacking in Europeans (Macalister); in the negro once out of five in the individuals (Eggeling). I have always found it in my material.

M. nasalis

In general this muscle appears to be as well developed in Mongolians as in Europeans. The development of the pars transversa (figs. 1 to 6 and 8) fluctuates widely. Thus size and shape of the nose are no criteria for the development of this muscle. According to Eggeling and Loth, it is well developed in the negro. Measurements of the greatest width of the pars transversa follow:

Negro: 12.5 mm. (Flower and Murie); 15 mm. (Loth).

Japanese: 12 mm. (Kudo).

Chinese: Left, 10, 10, 12 mm. (Kudo).

Europeans: Left, 10, 9, 12 mm. (Kudo); 11 mm. (Chudzinski).

Muscles of the two sides which meet in the middle line on the bridge of the nose have been observed in few instances in the negro, while I have met this connection frequently in Japanese. This muscle farther shows an intimate relation to other muscles in the yellow races. Frequently (eight times in fifteen half faces of Japanese) it passes over into the superficial fibers of the *M. procerus*. Eggeling has found the same in two cases out of five Herero heads. The muscle is also connected laterally with the *caput angulare*.

I find the pars alaris weakly developed, and frequently it could not be distinguished. Still it is possible that it had been removed in the preparation. According to Macalister, this part of the muscle can be absent in Europeans.

M. frontalis (figs. 2, 3, and 5 to 8)

Several authors have described a strong development of this muscle in negroes and Hottentots (Eisler, Loth, Fetzner). It is also well developed in the Japanese. The following measurements give an idea of the extent of this muscle:

1. The depth of the muscle fibers in the middle line, from the base of the nose outward

a. Measured as one banded group:

Japanese 81.8 (73 to 122) mm. (Kudo).

Chinese: right 75, 77 mm.; left 75 mm. (Kudo).

European: left 50 mm. (Kudo); 82.5 mm. (Chudzinski).

Negro: 78 mm. (Loth); 78 (62-93) mm. (Chudzinski).

b. Measured in a curve:

Japanese 76.1 (50-100) mm. (Kudo).

Chinese: right 71, 71 mm. (Kudo).

European: left 47 mm. (Kudo).

Negro: 60 mm. (Eggeling's 4 Hereros); 50 mm. ? (Livini);
57 mm. (Loth, 2 negroes).

The muscle is shortest in the median line and becomes increasingly deeper laterally so that the passage of muscle fibers into the galea forms an obliquely arcuate line (figs. 2, 3, 6, and 7).

2. The greatest breadth of the muscle, about perpendicular to the course of the fibers, is about 55.4 mm. in the Japanese.

A complete separation of the muscle into two halves has never been recognized in the Japanese and Chinese, likewise not in seventeen Hottentot heads, according to Fetzner.

M. procerus nasi (figs. 1, 2, 3, and 5 to 8)

This muscle is always well developed in the Mongolians; in the Europeans it may be occasionally wanting in one or both sides (Harrison, Macalister, Le Double). In the Japanese there is occasionally a considerable inequality of antimeric muscles. The passage of the muscle into the frontalis is the rule in Mongolians according to the literature, a complete separation appears opposite the frontalis; but in the European it seldom occurs. The facts in the case of the negro are not clear. In the Mongolians the superficial fibers of the muscle may reach the pars transversa of the *M. nasalis* farther up on the nasal cartilage, as is true of the negro, according to Loth. The direct junction of the procerus with the caput angularis, which seldom occurs in Europeans (Eisler), has been observed only twice in negroes (Loth), while I have come across it four times in the Japanese.

The antimeric muscles are hard to separate in the Mongolians. According to Chudzinski this muscle in negroes is on both right and left sides.

The breadth muscle of the two sides amounts to 10 mm. in Japanese; the smallest breadth is as follows:

Japanese: 6.6 (4 to 10) mm. (Kudo).

Chinese: right 4, 6 mm.; left 4, 5 mm. (Kudo).

European: left 3, 3 mm. (Kudo); 70.4 mm. (Chudzinski).

Negro: 5.4 (4 to 9) mm. (Chudzinski.)

M. occipitalis (figs. 1, 2, and 4 to 8)

This muscle is represented in Mongolians by a strong, coarse-bundled plate, which has varying outlines—often elongate quadrangular, more commonly triangular or lunate or somewhat notched. Chudzinski has not given accurate details for his material as to the measurements of this muscle. I have taken measurements as follows:

Depth (in front⁶):

Japanese (Kudo): 25.1 (12 to 37) mm.

Chinese (Kudo): right, 19, 18; left 18, 21, 21 mm.

European (Kudo): right, 21, 14; left, 16, 20, 22, 27 mm.

European (Chudzinski): 21 and 22 (17 to 28) mm.

Depth (behind⁶):

Japanese (Kudo): 28.5 (16 to 46) mm.

Chinese (Kudo): right, 18, 23, 30; left, 23, 30, 23 mm.

European (Kudo): right, 18, 23, 30; left, 16, 24, 25, 24, 23 mm.

European (Chudzinski): 27.7 mm.

Greatest depth:

Japanese (Kudo): 34.6 (23 to 53) mm.

Chinese (Kudo): right, 33, 37; left, 31, 33, 36 mm.

European (Kudo): right, 29, 38, 33, 46; left, 29, 35, 31 23 mm.

European (Chudzinski): 32.6 mm.

Negro (Chudzinski): 37 (34 to 47) mm.

⁶ I have measured the depth of the front and hinder sections of the muscle at about 5 mm. from the marginal bundles of either side and parallel to the course of the fibers.

Upper breadth⁷:

Japanese (Kudo): 63.6 (50 to 78) mm.

Chinese (Kudo): right, 76, 67; left, 64, 85, 66 mm.

European (Kudo): right, 63, 64, 77; left, 64, 75, 68, 56, 66 mm.

Negro (Chudzinski): 71.4 (61 to 81) mm.

Lower breadth⁷:

Japanese (Kudo): 56.3 (53 to 72) mm.

Chinese (Kudo): right, 64, 90; left, 61, 65, 86 mm.

European (Kudo): right 56, 60, 68; left 52, 53, 60, 45, 73 mm.

European (Chudzinski) 65 mm.

Negro (Chudzinski): 63.8 (43 to 96) mm.

As a vestige of the auriculo-occipitalis, which is well developed in apes, the anterior section of the occipital fibers toward the ear (namely, the *M. auricularis posterior*), shows a changing condition. In the posterior position of the muscle bundles are arranged more nearly vertically than in the anterior portion, and, directed obliquely upward and forward, paralleling each other, lie close together. The more anterior bundles, which only in a single Japanese (JX) course almost vertically, incline strongly forward. They are almost transverse in five cases (fig. 1), even obliquely ventral (in a Chinese and a European, fig. 8). In a Japanese head the anterior muscle part is separated by an interruption of continuity from the more posterior one.

The anterior muscle bundles run not only parallel with the upper margin of the *auricularis posterior*, but even fuse with it (in CII, JIX, and JI). In JIII such bundles become entirely separated from the hinder element and reach the ear (fig. 5). In Europeans this muscle usually unites with the *M. auricularis posterior* (Le Double). Eisler, on the other hand, does not confirm this for adults.

In seven out of 100 Europeans Austoni saw the median bundle of the occipitalis running dorsad to and parallel with the pos-

⁷ The distances, respectively, between the upper and lower ends of the marginal bundles of the two sides. •

terior margin of the auricularis. In negroes, Eggeling, who never observed an approximation of occipital fibers and the auricularis posterior, found a close approximation of this muscle to the auricularis superior (three cases in five Herreros). The occipitalis fibers which reach the auricularis were often found (three times) in Japanese (fig. 5) and in two Chinese (fig. 7); in JI they extend under the latter muscle.

In the negro (Chudzinski, Popowsky) the anterior bundle reaches the otic conch. According to Eggeling, these bundles are continued to the conch, sometimes by distinct, sometimes by weakly developed tendinous strands. In a Japanese (JI) and a European head (EV) I found the muscle fleshy, even till it reaches the ear cartilage.

M. transversus nuchae (figs. 1, 4, and 7)

This muscle has many variations; in text-books and atlases it is variously figured. Two types, however, may be distinguished.

The first type, the transversus nuchae (fig. 13) is a derivative of the auricularis posterior, that is, a median part (posterior) of the auricularis which becomes interrupted by the intermediate tendon. This type is always connected with the auricularis bundles by means of transverse tendinous fibers.

The second type (*M. corrugator posticus* Santorini, figs. 4 and 8) has no direct genetic connection with the muscles of the ear. It is the residue of the platysma fibers which radiate posteriorly into the neck region. The ventral end of the fibers may connect directly with the principal bundle of the platysma or may be separated from the latter (Pabis and Ricci).

Thus two types may always be readily distinguished. The muscle of the second type lies under that of the first, and, surrounded by thick, felted subcutaneous connective tissue, runs more obliquely caudad to the fascia parotida (*M. occipitoparotoidea* Chudzinski) or to the platysma. In the Mongolians it never arises directly nor by tendinous connections from the bones, but the fiber ends diverge more or less over the fascia of the neck. These differences of the two types are especially evident in the preparations which possess the muscles of both types, as I have observed in two individuals.

I have found the first type seven times in the Japanese (on an average, the greatest length is 23.7 (11 to 32) mm.; greatest breadth is 10.6 (4 to 18) mm.) and once in a Chinese (CII, greatest length is 10 mm., greatest breadth is 4 mm.) In Europeans Schulze describes eighteen cases in twenty-five individuals, Macalister seven in thirty individuals (23.3 per cent); but the conclusions of the latter have not been recognized by Knott and Le Double. The second type is rarer than the first; I found it three times in the Japanese, in one Chinese, and in one European.

In a Japanese (JVI) the transversus fibers, which probably belong to the first type, course along the posterior margin of the linea nuchae suprema ventromedial; at times tendinous platysma fibers are demonstrable, which show no connection with the platysma.

The relative frequency of the muscle in both types is about as follows:

JAPANESE (KUDO)	CHINESE (KUDO)	EUROPEAN (LE DOUBLE, ETC.)	NEGRO (CHUDZINSKI, ETC. ¹)
14 7 (50%)	3 1	89 33 (36.7%) ²	24 individuals 14 (58%)

¹ Chudzinski, Turner, Hartmann, Papowsky, Eggeling, Loth.

² LeDouble, Macalister, Schulze.

According to the number of half heads:

JAPANESE (KUDO)	CHINESE (KUDO)	EUROPEAN (LE DOUBLE, ETC.)	NEGRO (CHUDZINSKI, ETC.)
14 half faces 7 (50%)	3 half faces 1	118 half faces 48 (40.7%)	34 half faces 19 (56%)

From this it is evident that, based on per cent, there is a radical difference between the colored and white peoples.

M. auricularis posterior (figs. 1, 2, and 4 to 8)

This muscle is rather well developed in Mongolians and occurs in all of my material. Entire absence of this muscle is very rare in Europeans (Macalister, Le Double). The insertion on the

eminentia conchae by a long or short tendon always occurs. The origin lies behind the ear, along the neck line for a varying distance. The greatest breadth of the longest belly averages 0.5 mm. in the Japanese. The greatest length of the same belly is as follows:

Japanese (Kudo): 14 half faces, 30 (5 to 60) mm.

European (Kudo): right, 29; left, 26, 25, 38, 22 mm.

Chinese (Kudo): left, 36, 42 mm.

Negro (Chudzinski): 41.1 (23 to 46) mm.

Negro (Eggeling, Popowsky, Loth) 11 half faces 40 (23 to 46) mm.

The auricularis posterior may reach to the protuberantia occipitalis externa by evident growth. This apart from a consideration of the separation by an intermediate tendon into auricularis posterior and transversus nuchae.

With reference to the well-known division of the muscles into several parts (figs. 1, 2, and 4 to 8), I have grouped them as follows:

	1. SINGLE-BELLIED MUSCLE	2. DOUBLE-BELLIED MUSCLE (FIGS. 2, 4, 5, AND 8)	3. TRIPLE-BELLIED MUSCLE (FIGS. 1, 6, AND 7)
Japanese (Kudo) 14 half faces.....	6 (42.9%)	6 (42.9%)	2 (14%)
Chinese (Kudo) 13 half faces.....	1	1	1
European (Kudo) 5 half faces.....	1	2	2
Negro (Loth) 30 half faces.....	12 (40%)	11 (36%)	30 (25%)

The entire muscle generally possesses a broad, thick belly; divided muscle parts often unite with one another at their origin (fig. 8). I find a similar condition in the five Europeans which I have studied; also in the Japanese.

M. auricularis superior et anterior (figs. 2, 3, and 5 to 8)

All authors agree that these two muscles are incompletely separated from each other in negroes. In general, in the yellow race as in Europeans, I find a deep, broad, thin muscle plate which may join the occipitalis dorsally, the frontalis in front (figs. 2, and 5 to 7). On the whole, no peculiar differences between the white and yellow races are demonstrable in my material

M. auricularis inferior (Le Double)

I have never been able to find anything like the so-called *M. auricularis inferior* in Mongolians. Of course, I have occasionally observed a portion of the platysma which runs close below the ear conch.

M. auriculo-frontalis Gegenbaur (figs. 3 and 5 to 7)

I have found this muscle distinct six times in fifteen Japanese (fifteen half faces), besides twice in Chinese (three half faces). The extent of the muscle varies with the individual. In complete development (in a Japanese) it appears as a single muscle plate over the temple and crown, arising in front from the orbicularis oculi, and uniting behind with an *auricularis superior* (fig. 6).

In two cadavers (a Japanese and a Chinese) the auricular bundles radiate fan-like on the lower frontal section (fig. 3). As a rule, the thin pale muscle runs, with parallel fibers, over the temple and loses connection with the *auricularis* (fig. 5).

The *M. auriculo-frontalis* has been found in six out of thirteen negroes (Chudzinski, Eggeling, Popowsky, Loth) and in two out of seventeen Hottentots (Fetzer). In Europeans only Ruge states that the muscle occurs 'nicht ganz selten.' Sappey considers it constant, since, on temples apparently free from muscles, the microscope still demonstrates muscle bundles; but he says: "Mais sa minceur est extrême, et telle, que huit fois sur dix c'est à peine si l'on peut le distinguer à l'oeil nu."

2 THE FACIAL MUSCULATURE AS A WHOLE

Before considering the facial musculature as a whole, I desire to give the following short résumé of the literature relating to this subject.

In the negro (better, black race) the superficial muscles of the head, according to Chudzinski and others, are strong and greatly developed. Giacomini (cited by Loth), on the other hand, is the only one who has not expressly mentioned the primitive character of the facial muscles of the black races.

On the basis of the examination of four Hereros and a Herero child, Eggeling says that the relatively frequent presence of certain features, which have been regarded by Ruge as primitive, and the similar coincidence of several such characters in the same half faces distinguish the facial musculature of the Hereros from that of the European. Loth, who has compiled the scattered literature on the muscle system of various blacks, with reference to his own observations on the negroes, demonstrated a tendency toward a fusion of the single muscles or the formation of an almost united muscular layer in the face, a conception which has been confirmed by most of the students of the negro (Hamy, Chudzinski, Popowsky, Eggeling). Eckstein worked on the muscular system of a negro foetus, Hans Virchow on the facial musculature of sixteen negroes; the latter author states that the muscles of the negro are always more strongly developed than those of the stronger Europeans, are inclined to stratification, and that the fibers are coarse and are not exactly parallel.

As for the Papuans, the facial musculature of two newly born individuals studied by Forster constitutes "*das klassische Beispiel atavistischen Zustände.*" He mentions a stronger development, a very plump appearance, yet often no plain separation of single bundles to form special muscular entities, which could cause the delicate shades of facial expression. Fischer also finds in two adult Papuans an essential agreement with Forster's newly born individuals. Steffens and Körner, studying a newly born Papuan, contradict much of Forster's account which he regarded as *pithecoïd* in character.

In the Hottentots, as the result of Eggeling (a child) and Fetzner (seventeen individuals) show, the muscles of the face, lying between and around the mouth and eyes are somewhat bulky and undifferentiated; the single units are not so isolated and not so widely separated from each other as we are accustomed to see in Europeans. He declares, with apparent probability, that here the type of facial muscles corresponds to a lower grade of development of the human race.

In the Mongolians Birkner has already shown that his three Chinese heads are distinguishable from those of Europeans by

a more restricted division of the facial musculature. In his investigations on European, Chinese, and Japanese heads (the first two on which have been used by me in this study) Adachi has recognized two types of facial musculature: 1) All facial muscles strongly developed, coarse-fibered, and criss-crossed; 2) the facial muscles weakly developed, finely fibered, and little criss-crossed. He found, however, no special racial difference and only states that the first type usually appears in the broader-faced forms and the second in the narrower faces.

In the Europeans the head muscles have been exhaustively investigated from many angles. Since the results have, for the most part, not been given on a per cent basis, it will not do, in the search for racial anatomical differences, to evaluate the contributions of literature directly as a basis of comparison.

The results reached by my own investigations make it impossible for me to set up a single conclusive racial difference between Mongolians and Europeans. However, I will not deny simply on the ground of facial musculature that racial differences are present. I find differences between the Mongolians and Europeans which cannot well be explained as pure individualities. If, for example, one compare figure 7 (Chinese) and figure 8 (European), it is evident at first glance that in the former the face is strongly muscularized and little differentiated; in the latter, on the other hand, it is delicately built and well differentiated. The fact that these two chosen extreme cases belong to two different races may not be entirely casual. Among the fifteen Japanese neither the case present in figure 7 nor figure 8 is found. I have observed cases similar to those above cited very often. At least, it is an extreme case when the two conditions cited have not been found.

Also, in a general consideration of the musculature of the face as a whole, it is not too venturesome to assert that the Japanese and Chinese are separated from the Europeans by a somewhat smaller differentiation; that is, a tendency of single muscles to fuse superficially into a single plate; also, by greater development and greater extent of the musculature, just as other students have proved for the black race and for others.

This tendency, it seems to me, is stronger in the Chinese than in the Japanese. I found a highly developed crossing or extreme radiation of muscle fibers in two Chinese and in a few Japanese but never in Europeans. Perhaps the described differences would all be sharper if the comparisons of the musculature had been carried out on more abundant material.

In a strongly muscularized head the muscles are usually coarse-bundled. Nevertheless, on a basis of fineness or coarseness of bundles alone, I could not base any racial difference. When the thickness of the muscle bundles and the differentiation of single muscles, etc., are taken into account, the racial differences are insignificant in my material.

With reference to single variations among Mongolians, all types of varieties are found which are weakly expressed in the facial muscles of the Europeans; on the other hand, no varieties are shown in the Mongolians which have not been described for the Europeans. But we find that the presence of certain varieties or characteristics which manifest themselves only occasionally or seldom in Europeans or negroes, are observed regularly in the Mongolians.

In conclusion, I sum up the observations on the separate regions and the arrangement of the entire facial musculature of fifteen Japanese, three Chinese, and five European heads as follows:

1. The platysma which takes part in the structure of the cheek region, consists, for the most part, in the Mongolian of a continuous muscle plate, the same as in Europeans. Well-developed platysma fibers which extend in a line drawn from the corner of the mouth to the outer ear opening or course above it have been found in five Japanese and two Chinese.

Most of the cases of the aberrant platysma strands, which rise orbitotemporalward and may often reach the zygomaticus or orbicularis oculi, have been observed in the Japanese (eleven out of fifteen half faces), and constantly in the Chinese. I have nothing special to contribute with respect to the frequency of the neck portion in the Mongolians.

The *M. mandibulo-marginalis* has been found twice in fifteen half faces of the Japanese. It is rarer in Europeans.

2. The muscles of Mongolians (Japanese and Chinese) which function as dilators of the mouth appear to be less divided than in Europeans. In the Mongolians the muscles are generally difficult to distinguish from one another, are more extensive and coarser. In the Mongolians the triangularis fibers, for the most part, are spread out, fan-shaped, along the margin of the jaw.

The *M. risorius* is generally present in Mongolians (twice in fifteen Japanese half faces, never in Chinese, 43 per cent in negroes, 33 per cent in seventeen Hottentots. The *M. transversus menti* also occurs frequently (without exception in fifteen Japanese and three Chinese, 60 per cent in Europeans, 30 per cent in the negro). In Mongolians, as a rule, the three parts of the *quadratus labii superior* fuse into a single plate; further, the *caput zygomaticus*, constantly present in Mongolians, is distinguishable with difficulty from the neighboring muscles.

3. The musculature around the eye is more strongly developed in Mongolians (especially in Chinese), as I have found by comparison with five Europeans. The bundles radiating at the lower median part are especially strongly developed. The separation of the muscle from its surroundings is usually not definite (connection with the *M. zygomaticus* and *M. quadratus labii superioris*).

4. The *epicranius* shows no noteworthy difference between Japanese and Europeans. The junction of the muscle of either side along the median line in the region of the middle third of the muscle follows the same plan as that in the European (and also in the negro).

5. Likewise, I find no special difference in the muscles in the vicinity of the conch in my material. Nevertheless, it might be desirable to undertake an investigation of the ear muscles in more extensive material, in which eventually a racial difference might be discovered.

It may be noted that the *M. transversus nuchae* is more frequent in the Japanese (negro 58 per cent, Japanese in half of the cases of half faces, Europeans 37 per cent). The (*M. auriculo-frontalis* was found six times in the Japanese (fifteen half faces) and once in the Chinese (three half faces).

6. In spite of a considerable difference in form of the nose, nothing noteworthy has been found with respect to the muscles.

7. The facial musculature as a whole in the Mongolians appears to show only individual minor differences.

Thus, in general, the facial musculature of the Japanese presents a more primitive type than that of the European. It is to be noted, however, that in certain parts, the reverse holds.

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EXPLANATION OF PLATES 1 TO 3

Comparison of the three plates (1, Japanese; 2, Chinese; 3, European) shows at the first glance great differences in the facial musculature as a whole. In the Japanese and Chinese the facial muscles are strongly developed, coarsely bundled, spread widely, with a tendency toward a fusion of the separate muscles into a single sheet, often (especially in the Chinese) radiated and interlaced. In the European (pl. 3), on the other hand, the separate muscles are delicate and well differentiated. I selected extreme cases for my figures of the different races; naturally, such great differences are not universal. The separate muscles are described in detail as follows:

PLATE 1

EXPLANATION OF FIGURE

6. Female Japanese XI, twenty years old. Platysma forms an entire plate, the pars aberrans surpasses the mouth angle-auditory meatus line in a fan-like arrangement. The triangularis is broad at its origin. Between this muscle and the risorius (the latter consists of two bundles) are intermediate marginal fibers. It is noticeable that several isolated fiber strands of the M. mandibulomarginalis, partially covered by the triangularis, run obliquely on the platysma. The coarsely bundled zygomaticus is scarcely separated from the caput zygomaticus and the orbicularis oculi. The superficial layer of the caninus, at the insertion of the zygomatic, consists of weak fibers. The caput angulare of the quadratus labii superioris is strong. The lateral marginal bundles of the orbicularis oculi are compact and pass over below to the zygomaticus. Also there are laterally directed radial bundles on the lower lateral quadrant of the orbicularis. The medial lower bundles are compact and somewhat swollen. The M. frontalis is relatively fine-fibered. The lateral part of this muscle, the auriculofrontalis and the auricularis superior and anterior come in contact with each other and form a thin connected sheet over the temple. The medial vertical fibers of it, along the temporal vessels, are bent toward the ear; the fibers of the auriculofrontalis which run forward, end on the lower region of insertion of the frontalis, beneath the orbicularis oculi. The three parts of the M. auricularis posterior are not sharply separated from each other.



PLATE 2

EXPLANATION OF FIGURE

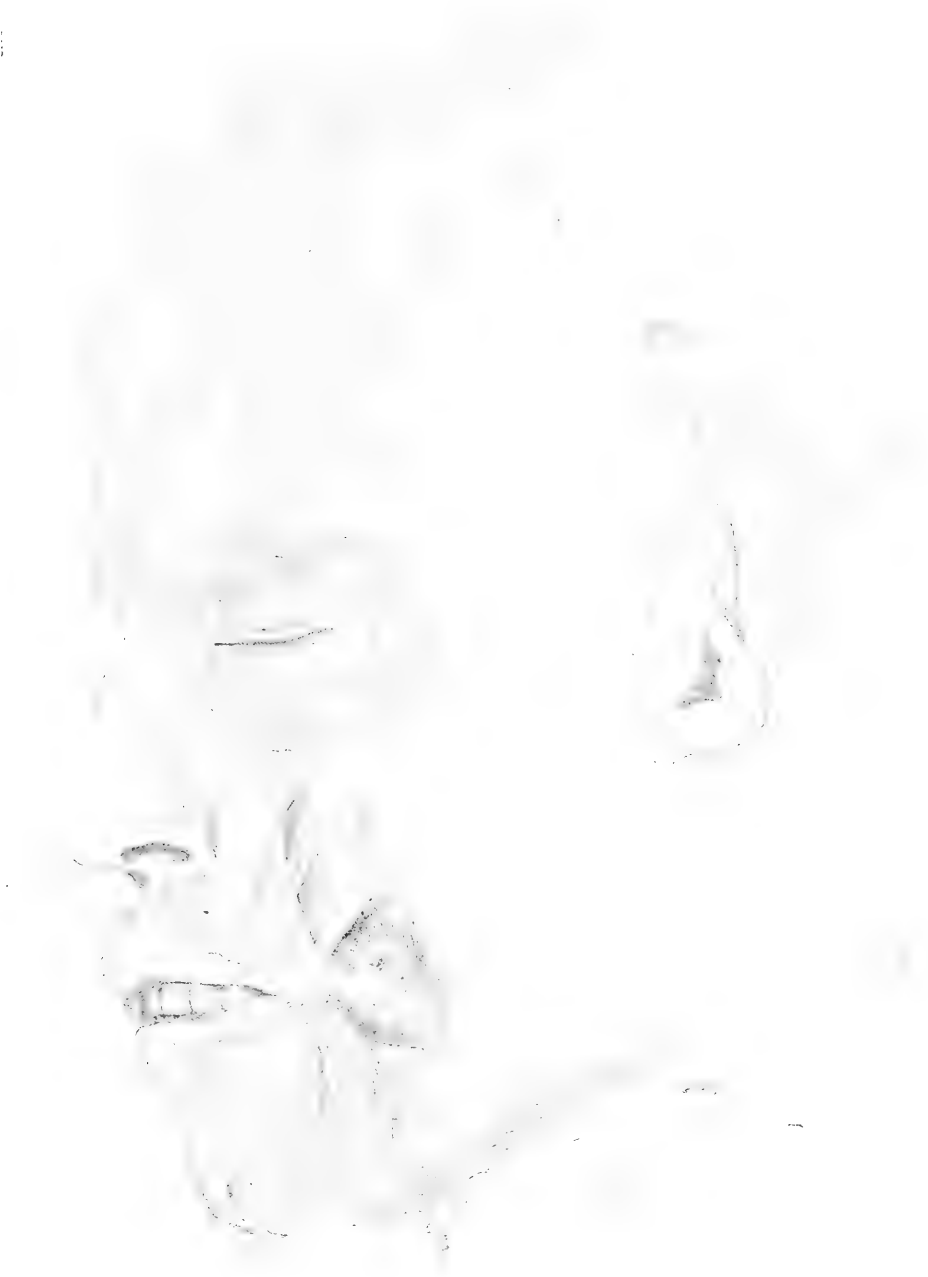
7. Male Chinese II. The platysma is coarsely bundled and forms an entire plate. Its upper part extends above the mouth-angle meatus line toward the zygomatic; the inferior part passes under the triangularis. The triangularis, together with the *M. risorius*, forms a fan-formed muscle mass; some intermediate bundles between the two muscles extend to the platysma. At its origin the zygomaticus is overlaid by and partially interlaced with the orbicularis oculi. Strong radiating and often crossing bundles on the lateral part of the orbicularis oculi are striking. Marginal bundles from the auriculofrontalis radiate upward; the curved lateral fibers interlace with ascending platysma fibers. Those running ventrad extend farther on the zygomaticus, even to the quadratus labii superioris. The lower medial bundles are also well developed; the superficial layer is transected in the illustration and the deep portion passes under the lateral marginal bundle and then interlaces with the cervical part of the platysma. The laterally directed marginal bundles of the orbicularis oculi lie deep between it and the ear, and are visible in the plate through an artificial opening. The *M. frontalis* is connected laterally with the higher auricularis bundles. The auriculofrontalis runs between the two muscles, joins the auricularis anterior, and continues forward and upward to the lateral part of the frontalis. The temple is also well muscularized. The extent of the occipitalis is noteworthy; the posterior bundles are more vertical and the anterior incline more forward and reach the auricularis superior. At last they unite transversely with the auricularis posterior. The auricularis is inclined forward and downward and consists of four parts, not readily separable. The cervical part of the platysma is broadly extended under the ear, the hinder limb approaches the median line above the insertion of the sternocleidomastoid; the anterior diverges at the hinder part of the cheek region of the platysma. Some bundles of the latter ascend further and cross the diverging bundles of the orbicularis oculi.



PLATE 3

EXPLANATION OF FIGURE

8. Male European II. The platysma runs forward as an entire plate, but lacks a pars aberrans. The triangularis is small. The zygomatic is entirely separate from the orbicularis oculi and the caput zygomatici. The three heads of the quadratus labii superioris are well differentiated. The orbicularis oculi forms a closed ring around the eye, diverging bundles are present. The frontalis extends far toward the vertex. There is no musculature on the temporal region. The auricularis superior and the occipitalis are moderately developed; the latter does not reach the ear and the occipitalis. The auricularis posterior consists of two parts, connected with each other at the origin.



Resumen por el autor, Clarence Lester Turner.

El ciclo estacional en el espermario de la perca.

El presente trabajo es un estudio de la variación volumétrica del testículo de la perca. El tamaño mínimo se encuentra a principios de verano. El comienzo del aumento de tamaño es brusco y este aumento es muy rápido, alcanzando el máximo de tamaño en la última parte de Noviembre en la cual el peso de los testículos es 60 veces mayor que su peso mínimo. El volumen del espermario decrece a consecuencia de la puesta (desde primeros de Marzo hasta últimos de Mayo). El cordón de células germinativas que suministra anualmente elementos al testículo está situado centralmente a este órgano; las células germinativas emigran dentro de este último, siguiendo exteriormente las paredes de los lóbulos y alojándose en los lóbulos de la periferia. A partir de esta, el testículo está ocupado por células germinativas, que se transforman en espermatogonias, las cuales forman cistos. El comienzo del aumento de tamaño del testículo coincide con la formación de las espermatogonias. No hay periodo de crecimiento antes de la maduración. La espermatogénesis tiene lugar en los primeros meses del otoño. El comienzo de la disminución anual de temperatura (a últimos de Agosto) coincide con el aumento volumétrico estacional del testículo. El comienzo de la disminución estacional del testículo (a primeros de Marzo) coincide con el aumento de temperatura en el agua.

Translation by José F. Nonidez
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THE SEASONAL CYCLE IN THE SPERMARY OF THE PERCH¹

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I. INTRODUCTION

The study of the germ cells in vertebrates has been confined principally to two lines of investigation. The first has undertaken to recognize the germ cells in the embryo and to follow their lineage, on the one hand, backward through the younger embryos to the first stages in which they may be positively identified as germ cells and, on the other, to trace them to the

¹ A Thesis submitted to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

² A part of this work was carried out in the laboratories of the Department of Anatomy and Biology, Marquette University School of Medicine.

stage in which the germ gland is first formed. The interest has centered principally about the location in which the germ cells apparently originated, in their active migration, in the paths traversed in reaching their final place of lodging, and in the number which entered the germ glands. Eigenman ('92, '96), Wheeler ('99), Dustin ('07), Beard ('00, '02), Woods ('01), Jarvis ('08), Allen ('06, '09), Dodds ('10), and Swift ('14) have all contributed to the early history of the germ cells in different vertebrates. Embryologists and histologists have contributed to the further history of the germ cells up to the time in which they become definitely placed in the fully developed germ glands.

The second line of investigation has been cytological. The problems of spermatogenesis—synapsis, chromosome number, the reduction process, nuclear and cytoplasmic inclusions, etc.—have so occupied the attention of the cytologist that he has confined his study mainly to the testis of the sexually mature animal or to those stages in the history of the testis which furnished satisfactory cytological material.

Among the vertebrates are some forms which have lent themselves admirably to the investigation of the origin of the germ cells and to cytological studies. Other animals, because of their small germ cells or the difficulty of obtaining complete embryological series have been abandoned by both the embryologist and the cytologist. Eigenmann and Dodds have given excellent accounts of the germ cells and their behavior in the embryos of teleosts. Many other papers have dealt with the fertilization and cleavage of the teleostean egg and the embryology in a number of teleosts has been described in detail. There is a dearth of literature, however, on the morphology of the adult teleostean testis and the germ cells in the adult have been investigated but little.

It is a familiar fact that the ovaries and testes of teleosts as well as those of amphibians and of some other vertebrates differ in size at different seasons of the year. Advantage has been taken of the volumetric variation in the ovary of the fish to work out some valuable data concerning the growth and dis-

tribution of fish at different seasons. So far as the writer can determine, however, no thorough study has been made upon the seasonal changes that take place in the teleostean testis.

It is the main purpose of this paper to describe the changes that have been observed in the testis of the yellow perch (*Perca flavescens*) during the different seasons of the year with reference to the gross and microscopical anatomy and the cytology.

II. MATERIAL AND METHOD

The material for this investigation was collected from Lake Mendota at Madison, Wisconsin, and from Lake Michigan at Milwaukee, Wisconsin, between May 1, 1915, and October 1, 1917. From five to twenty specimens were examined each week during this period. All were weighed fresh and the testes were weighed before fixation. Spermaries of other teleosts (carp, sunfish, crappie, pickerel, and lake trout), as well as those of *Necturus*, of two species of turtles, of several birds, and of the muskrat, were examined from time to time for comparison.

For histological study material was fixed in 10 per cent formalin and in a picro-acetic acid mixture. Haematoxylin and haemalum stains were used with an eosin counterstain. Resorsin-fuchsin was used to stain the elastic tissue.

In fixing material for cytological detail, Flemming's, Gilson's, and Bouin's mixtures were used, the best results being obtained with Bouin's mixture to which had been added a small proportion of urea (Allen, '16). Flemming's triple stain, safranin counterstained with licht grün and haematoxylin counterstained with eosin or licht grün were employed. Gentian violet alone also proved valuable. Sections from 5μ to 15μ thick were used in the histological work. Both smears and sections were used in the cytological study, smears being prepared between coverslips as described by Agar ('11).

The writer is indebted to Prof. A. S. Pearse for aid in collecting specimens in Madison, to Prof. M. F. Guyer for suggestions in technique and for criticisms, and to Prof. E. A. Birge, who kindly loaned a record which summarized the last fifteen years' data on the temperature of the water of Lake Mendota. Ac-

knowledge is also made to Mr. Leo Massopust, artist at the Marquette University School of Medicine, for instruction in his method of illustrating.

III. ANATOMY OF THE TESTIS

1. General relationships

The testes are two elongated white bodies situated in the posterior part of the body cavity just ventral to the swim bladder. They fuse together at their posterior ends, forming a single body. They are oval in shape and taper toward their posterior ends. Usually they are about equal in size, but cases have been noticed in which one testis was nearly twice the size of the other. They are attached to the swim bladder by two delicate mesorchia which converge posteriorly into one at the point where the testes fuse. Anteriorly the mesorchia extend beyond the limits of the testes and support a sheath containing the genital arteries and veins.

Posteriorly the testes communicate with the urogenital opening by a thin-walled but capacious sinus. A horizontal septum separates this sinus from a dorsal chamber which receives the common ureter from the Wolffian bodies. Both chambers terminate in the common urogenital opening.

2. Volumetric variation

The volumetric variation of the testis from one season to another is a conspicuous feature. Just after the spawning season, which occurs in late April or early May, the testis is depleted and its volume is slight. During May, June, July, and early August there is practically no change, except for a slight individual variation. Late August witnesses the initiation of a sudden increase in volume, which proceeds so rapidly that by the latter part of September the weight is between thirty and thirty-five times as great as it was six weeks earlier. By January the maximum weight has been attained, when it is nearly forty-five times as great as the weight of the depleted testis. At this time it represents from 4.58 to 5.9 per cent of the entire gross weight of the body.

The weight and size of the testis is proportionate to the weight and size of the body. For example, at one season of the year the weight of the testis of a fish weighing 97 grams was 5.25 grams while that of a fish weighing 40 grams was 2.25 grams. In the first case the weight of the testis was one-eighteenth, and in the second it was one-seventeenth of the gross weight of the body. While the actual weight of the testis cannot be taken as a reliable criterion, therefore, for the volumetric variation of the testis, the proportion between the weights of the body and of the testis will be fairly constant for a large number of individuals for any given season. Hence, in constructing a curve to show the variation in the volume of the testis in different seasons, the proportion which the weight of the testis forms of the total body weight has been taken as the unit by which points may be determined for a curve. Figures 5-22 represent a series of camera-lucida drawings showing the variation in size and in shape during the different seasons. Figure 7A represents the variation graphically, and the accompanying table gives the figures upon which this curve was based.

DATE	AVERAGE WEIGHT OF FISH	AVERAGE WEIGHT OF TESTIS	AVERAGE RATIO BETWEEN TESTIS AND BODY WEIGHT
	<i>grams</i>	<i>grams</i>	<i>per cent</i>
July 1.....	78.4	0.1	0.12
July 15.....	68.2	0.1	0.14
August 1.....	73.1	0.1	0.14
August 15.....	72.48	0.177	0.21
September 1.....	89.7	0.37	0.47
September 15.....	60.98	0.726	1.19
October 1.....	72.00	2.46	3.44
October 15.....	78.2	4.15	5.32
November 1.....	75.5	4.44	5.88
December 1.....	84.00	4.2	5.00
January 1.....	67.5	3.21	4.76
February 1.....	72.6	3.16	4.34
March 1.....	91.4	4.15	4.54
April 1.....	75.3	2.78	3.70
April 15.....	68.8	2.86	4.16
May 1.....	76.7	1.91	2.50
May 15.....	72.3	0.84	1.62
June 1.....	81.4	0.26	0.338
June 15.....	85.00	0.15	0.17

The spaces between the ordinates represent intervals of approximately thirty days while the spaces between the abscissae represent variations of one per cent in the ratio between the weight of the testis and the weight of the body.

The maximum weight of the testis is reached about November 1. From November 1 to April 1 there is a gradual decline in weight. The irregularity in the curve between December 1 and March 1 is occasioned by individual variation. During these months difficulty was experienced in getting specimens, and it is probable that there would have been no irregularity had enough material been at hand. The declining curve from March 1 to July 1 does not indicate that there is a gradual expulsion of the spermatozoa by each individual between these dates, but rather that a few fish discharge their spermatozoa as early as March 1 while others do not discharge until late in May. The curve declines as the proportion of those which have discharged increases. Although the point in question was not verified by actual observation, it is probable that each fish has a series of discharges, for a great many testes showed different stages of depletion during the spawning season.

No attempt was made to keep separate data for fish of different ages when it was found that specimens varying in weight from 60 grams to 300 grams did not offer any essential differences from the general course described.

3. Microscopical anatomy

The testis is a sack enclosed in a connective-tissue sheath which contains a large proportion of elastic elements. Within each testis, on its ventral side, is a connective-tissue core, deeply imbedded, from which septa radiate. These septa extend to and join the testis-wall, dividing the entire organ into lobules. This connective-tissue core is somewhat comparable to the mediastinum testis of the higher vertebrates. The cores of the two testes converge and fuse posteriorly at the point of fusion of the testes. The elastic fibers are very abundant throughout the entire testis. Two heavy cords of elastic fibers are located in the mesorchia which suspend the testes from the swim bladder.

They are fused together for a short distance at the point of the fusion of the testes, but they redivide, one cord passing into the connective-tissue core of each testis and, subdividing within, send one branch anteriorly and one posteriorly. Small branches extend into each of the connective-tissue septa in the form of

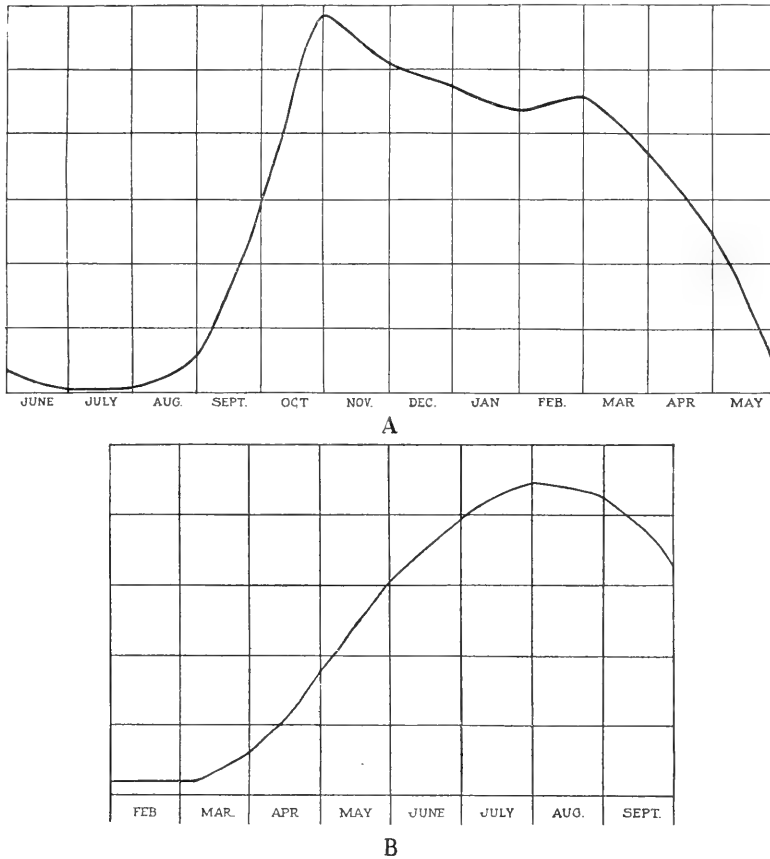
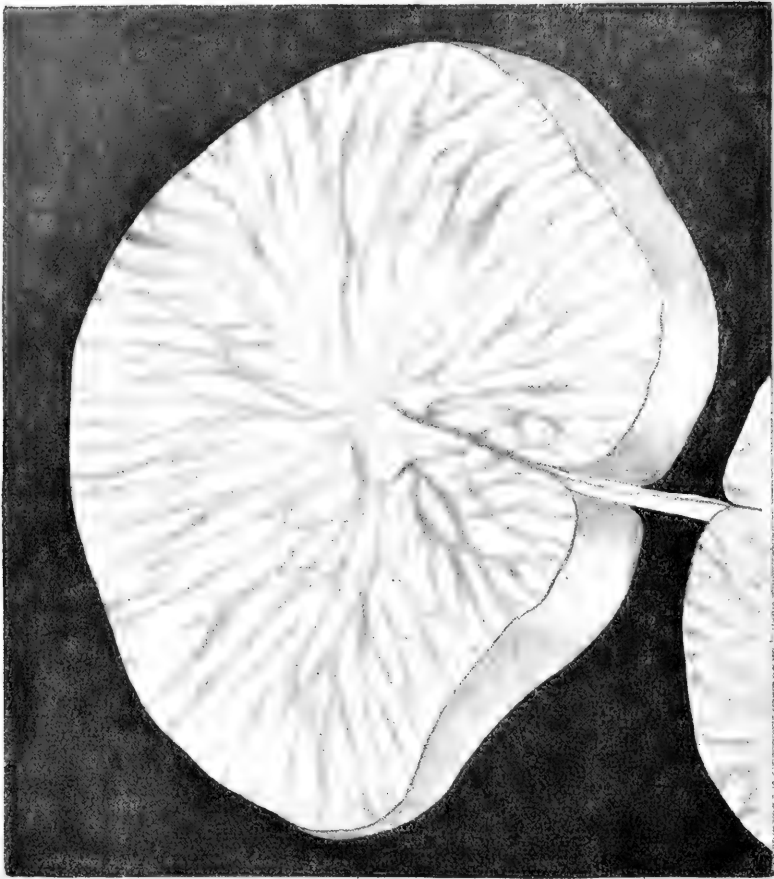


Fig. 1. A. Graphical illustration of volumetric variation in testis. Each space on the ordinate represents a period of one month. Each space on the abscissa represents a variation of 1 per cent in the ration between the body weight and the weight of the testis. B. Graphic representation of the seasonal variation in temperature in the water of Lake Mendota at a depth of 6 meters. Each vertical space represents a variation of 5° . Each horizontal space represents a period of one month.



A



B

2



C

Fig. 2. Sketch illustrating casts of lobules. A. Segment showing lobules in place. B. Surface view of a group of lobules. C. Side view of same group of lobules.

cords and bands. The amount and distribution of the elastic tissue is important, since, in the absence of muscle fibers and of special ejaculatory organs, it must play some part in the expulsion of the spermatozoa. The reduction of the testis to its normal small size after its distention must also depend upon the elastic tissue.

The genital arteries and veins passing into the connective-tissue core at the anterior end of the testis give off minute branches into the testis as they proceed posteriorly. Elongated bodies of adipose tissue are also located in the connective-tissue core of the testis and send out digitate processes into the septa between the lobules.

The lobules are irregular-shaped spaces with their apices at the center of the testis and their broader ends directed toward the periphery. The precise shape can be obtained in the form of casts by allowing a testis to soften for a time, fixing it in alcohol and then tearing it apart. The hardened mass of spermatozoa, representing a cast of the lobule, may then be dissected out with ease (fig. 2 *a*, *b*, and *c*). The same procedure may be carried out with success in testes which contain only the early, transforming germ cells. The casts of the lobules appear as flattened leaves, joined at their apical ends, diverging and branching toward the periphery of the testis. As many as five branches may be given off from a single central trunk, and these branches may again bifurcate before coming into contact with the testis wall. Some of the branches end blindly. In the pickerel the branches of the tubules at the periphery of the testis are much more finely divided and are convoluted, giving the appearance of seminiferous tubules (fig. 23).

4. Discussion of seasonal histological changes and comparison with other animal groups

From the foregoing description it follows that the perch testis differs radically from that of the Sauropsida and the mammals. The fact, too, that the testis undergoes such a complete seasonal change places it in a class far removed from the amniotes. It

resembles the testis of the Amphibia in so far as there is a lack of seminiferous tubules and in the presence of lobules which become divided up into cysts as soon as the maturation process has begun. While the amphibian testis undergoes a seasonal change, there is no such seasonal variation in volume as there is in the perch. There is also an anteroposterior seriation in the testis of the urodele, while the testis behaves as a unit in the perch.

It is evident from only a cursory examination, however, that there are variations in different teleosts which differ considerably from the course of the changes which occur in the perch. After some germ cells have migrated to the periphery of the testis in the perch there is a proliferation which increases their number. There is also a constant addition occasioned by the arrival of new migrating cells. This process continues until there is a solid cord of germ cells which fills each lobule of the testis at its normal small size (early August). Some connective-tissue cells are found among the germ cells. The transformation of the germ cells into spermatogonia is contemporaneous with the beginning of the increase in the volume of the testis (late August). After spermatogonia are formed the process of spermatogenesis takes place rapidly and the volume of the testis increases apace. The spermatogonia arrange themselves into cysts which are imperfectly divided by connective-tissue cells. Each cyst behaves as a unit during the maturation process, all of the cells passing through the same stage at the same time (figs. 25 and 26). The cysts at the center complete their maturation and form spermatozoa first, but there does not seem to be any definite seriation from the center to the periphery of the lobule.

The formation of cysts within the lobules is not as clearly shown in the perch testis as it is in the testis of the sunfish (fig. 24). There is a close resemblance between the cyst formation in the testis of these teleosts and in the testis of many arthropods.

When the germ cells first become lodged at the periphery of the testis in the perch they form a lens-shaped mass which

conforms to the shape of the cavity at the peripheral end of the lobule. In the pickerel the peripheral ends of the lobules become subdivided into pockets which are long and tubular. When the germ cells reach these pockets in the pickerel they become arranged along the inner walls, leaving a lumen in the center. Because of the peculiar form of these peripheral subdivisions of the lobules and the relations of the germ cells to them, there is a resemblance between them and the seminiferous tubules of the higher vertebrates (fig. 23).

There is a close resemblance between the cords of germ cells formed annually in the lobules of the testis of the adult perch and the formation of cords of primordial germ cells in the embryonic testis of mammals. In both cases the cord is formed by germ cells which have migrated into the testis from without. Here the resemblance ceases, for a part of the germ cells in the cord of the embryonic mammalian testis are destined to form nurse cells, while in the perch all the cells give rise to sexual products.

There is a marked change in the somatic structures of the testis during the changes in volume, but this does not involve growth. The interlobular walls become thin and the wall of the testis decreases in thickness. The blood-vessels dilate, apparently to meet the needs of the rapidly dividing germ cells. There is no increase in the adipose tissue. The entire testis seems to be in a state of tension while it is at its maximum size, as evidenced by the stretched condition of the interlobular and testis walls and by the deflection at the peripheral ends of the branches of the lobules (fig. 2, c). When the spermatozoa are expelled the region nearest the connective-tissue core on the ventral side of the testis is depleted first. When all the spermatozoa have been expelled, the normal thickness of the interlobular walls and of the testis walls is restored and the small size of the testis is resumed.

It is interesting to speculate as to the character of the changes which occur in those teleosts which spawn but once in their lives. The Pacific salmon and the eel would furnish material for such an investigation.

IV. HISTORY OF THE GERM CELLS

1. *Resting stage and period of migration*

A cord of germ cells outside of the testis was found in a single specimen which was killed on May 5. Unfortunately, this was the only fish taken at this date and, though the cord has been sought in specimens taken at other dates, it has not been found. Consequently, further investigation of this point must be postponed until a time which will again furnish favorable material. In this specimen there were no less than 5400 germ cells by actual count. The cells, imbedded in a connective-tissue matrix, varied in size as well as in shape (fig. 27). Most of them were apparently at rest, although the irregular shape of some seemed to indicate migration. No dividing cells were found among them.

In these cells the cytoplasm is hyalin or reticular and stains very lightly. The nuclei vary in shape, some being spherical, others oval, and a few irregular, having a marked indentation on one side. The plasmosome is a conspicuous object and lies near the nuclear wall. Most nuclei contain a single plasmosome, but it is not unusual to find two. The chromatin is well distributed, being scattered along the linin threads and massed together in some places to form chromatin knots. The linin threads appear to be in contact with the nuclear wall and radiate from the region occupied by the plasmosome.

The actual migration of the germ cells from this cord is assumed upon the grounds of the following observations:

1. Germ cells are found in various locations at different periods.

- a. A mass of germ cells larger than any other to be found at that time occurs in the cord outside of the testis (fig. 27, fig. 31, a and b). The question as to whether there is a progressive depletion of the germ cells in the cord could not be settled because material was not preserved which would show this point.

- b. Germ cells of a peculiar shape are found along the septa of the lobules from the center to the periphery of the testis during the time in which clusters of germ cells are formed and increased at the periphery of the testis (fig. 32).

c. Clusters of germ cells occur at the periphery of the testis and their numbers are greatly increased at the time when the germ cells are found along the septum walls. Few mitotic figures occur, and it is evident that the original clusters of germ cells at the periphery do not give rise to all the cells found in this location a short time afterward.

2. Germ cells along the septa between the lobules have an elongated and an irregular shape which suggests amoeboid motion (fig. 28).

3. The germ cells in the region of the junction of the interlobular septa and the periphery of the testis (fig. 29) seem to show direct transitional stages between their migratory form and their resting form.

During the migration there seems to be a slight increase in the volume of the cells. There is no change in the character of the cytoplasm, but the nucleus is a little more hyalin owing to the more even dispersion of the chromatin along the linin threads.

After migration, the germ cells come to lodge at the distal end of the lobules. Some of the lobules do not extend entirely to the periphery of the testis, but end blindly a short distance from the center (fig. 2, c). In consequence, the germ cells, after their migration, occur in pockets some distance from as well as at the periphery. It seems that the tendency to migrate ceases only when the cells have definitely come into contact with an obstruction at the end of the lobule. This fact would seem to furnish evidence in favor of the germ cells accomplishing migration actively rather than behaving in a purely passive manner. During the migration most of the cells are found along the inside of the lobule, but some are actually within the intervening septa. Some recent work in tissue culture has shown that in cells cultivated in vitro, migration is facilitated by the presence of strands along which the cells may move. The strands of fibrous connective-tissue in the walls of the lobules would furnish admirable supports of this character. No mitotic figures were observed in the migrating cells and it is assumed that they do not divide during this period. In this connection it is sig-

nificant that germ cells in embryos generally do not divide while they are migrating.

Once the germ cells have become lodged they undergo an immediate change. A comparison of *a* and *b* in figure 3 indicates that there is a slight increase in volume. There is also the formation of some darkly staining spherules which accumulate around the nuclear wall and pass out into the cytoplasm (fig. 33). The actual formation and extrusion of these spherules is, of course, a matter of interpretation, but no spherules are found in the nucleus of the migrating cells except the plasmosome, and the cytoplasm is entirely free from them. Such a change might well be brought about by a change in the metabolism of the cell. During migration the energy would be consumed in locomotion, but when the cell becomes sedentary the energy would be diverted into growth and a reorganization of the cell contents preparatory to division.

There is a striking parallel between the behavior of the germ cells in the adult perch and the embryonic cells described for *Lophius* by Dodds ('10). He remarks as follows:

In all vertebrates examined, this period (of growth) corresponds to the time during which the germ cells are in active migration, and it has been suggested that possibly the energy of the cell is expended in locomotion rather than in growth and cell division.

The above discussion of conditions observed in these cells during the rest period offers no explanation why this period of suspended activity begins, nor why after a time it comes to an end. At its beginning, before there are any differences we can detect with the eye, there must be an unseen physiological difference which determines the future behavior of the cell. Whatever the nature of the difference, it is one of the earliest of which we have evidence in the cleaving egg of *Lophius*.

The migration occurring in the perch seems to correspond to the period of rest. Dodds has also called attention to the fact that the germ cell retains its embryonic character longer than any of the other tissues of the body. It is possible that the germ cells migrate seasonally because they have not differentiated, even in the adult perch, and that they are only fulfilling their innate tendency to migrate whenever the opportunity offers or when there is a proper stimulus provided. The problem en-

countered by Dodds as to why this period of migration and cessation from division should be inaugurated and why it should come to an end is also met in the present study. The point will be discussed further in another part of this paper. The behavior of the migrating germ cells in the perch would suggest, however, that the capacity for migration had not been exhausted when the embryonic germ cells had reached the germ gland, but that under favorable circumstances they might again undertake locomotion after a period in which their energy had been used in a static condition resulting in growth and cell division.

The amoeboid cells have been observed along the lobule walls shortly before the spermatozoa are discharged in April and continuously until the period of spermatogenesis, which begins about the first of September.

2. Period of proliferation and growth

This period extends from the time in which the migrating germ cells begin to collect at the periphery (early April) till they are transformed into spermatogonia (early August).

The transformation which the migrating germ cells undergo when they reach the periphery of the testis has already been described above. Active growth starts as soon as the cells are lodged, and by early May a small proportion have become considerably enlarged (fig. 3, *a* and *b*). The clusters at the periphery at this stage contain less than a hundred cells. By early July the clusters have increased considerably (fig. 30, *G.c.*) and there is a larger proportion of the larger cells (fig. 3, *c*). Proliferation is going on, but very slowly, and it is evident that the increase in the clusters is partly due to the arrival of new migrating cells. The only mitotic figures in which a definite chromosome count could be made are furnished by these larger cells (figs. 35, 36). Twenty-seven chromosomes could be counted distinctly. Nucleus and cytoplasm maintain their former volumetric proportion during growth, and the plasmosome also increases in size. The darkly staining bodies which appear at the edge of the nucleus when the cell first becomes lodged disappear during growth.

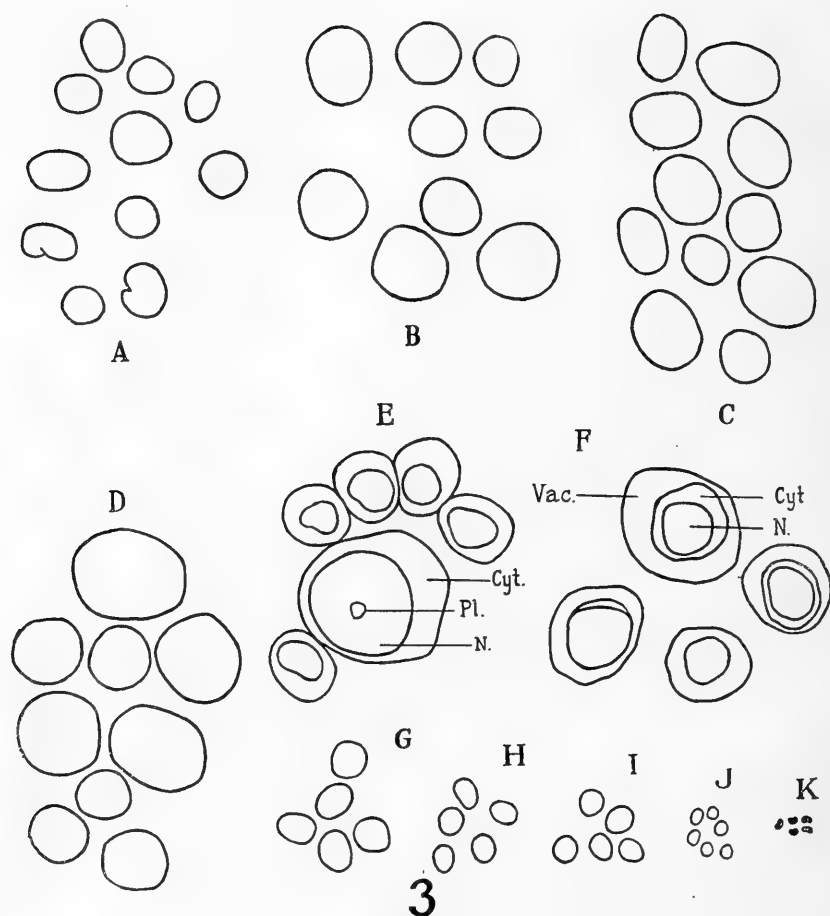


Fig. 3. Camera lucida drawings to show size relationships of cells during different periods. All figures except e and f represent outlines of nuclei and all are drawn to the same scale with an $\times 8$ ocular and 1.9 mm. oil immersion lens. A. Germ cells in cord outside of testis (May 5). B. Germ cells at periphery of testis (May 5). C. Germ cells during period of growth and proliferation (July 3). D. Germ cells during latter part of period of growth and proliferation. Maximum size of cells shown (August 5). E. Outline drawings illustrating comparative size of largest and smallest cells in a lobule, also volumetric relations of cytoplasm, nucleus and plasmosome; *cyt.*, cytoplasm; *n.*, nucleus; *pl.*, plasmosome. F. Drawing illustrating size relations during transformation of germ cells into spermatogonia; *vac.*, clear vacuole of liquid surrounding transforming cell; *cyt.*, cytoplasm; *n.*, nucleus. G. Group of spermatogonia. H. Group of primary spermatocytes. I. Group of secondary spermatocytes. J. Group of spermatids. K. Group of spermatozoa.

The germ cells come to their maximum size about August 5 (fig. 34, *a, b, c*). At this time the entire testis in its normal small size is filled with solid cords of germ cells (fig. 30, *G.c.*). About 5 per cent of the entire number have reached the maximum size; approximately 15 per cent are still very small and apparently have but recently migrated, while the remainder represent intermediate sizes.

The cytoplasm of the largest cells is reticular and is free from darkly staining inclusions. The nucleus is remarkably hyalin. The linin threads are attenuated and the chromatin is well distributed. In most cases the plasmosome is a large spherical structure which takes an acid stain, but in some cases the larger structure is absent and its place is taken by two or three smaller spheres. Figure 34 represents three cells taken from the same lobule, *c* representing a young germ cell before growth; *b*, an intermediate stage, and *a*, a cell at its maximum size.

Immediately upon reaching their maximum growth the cells are transformed into spermatogonia. The proportion of the largest cells never increases to more than 5 per cent and the transforming cells are few in number. This would indicate that the time occupied in the final stage of growth and in the transformation into spermatogonia is short. The smaller and the intermediate cells are growing meanwhile, and there is a continual procession of growth and transformation until late in November. During late September and October each lobule shows a profusion of young growing germ cells, of germ cells at their maximum size, of transforming germ cells, of spermatogonia and of all the succeeding stages of spermatogenesis, including the mature spermatozoa.

3. Period of transformation into spermatogonia

During the transformation into spermatogonia two features of the process are outstanding: 1) There is a definite reduction in size. 2) There is a change in the chemical composition of the cells as shown by its capacity to acquire a deeper stain.

Three stages of the transforming cells are shown in figure 37. In the first stage there is a contraction of the entire cell and probably the extrusion of a clear fluid. At any rate the boundaries which marked the limits of the cell at its maximum size are maintained, and there is a space between this boundary and the contracted cell, giving the appearance of a cell suspended in a chamber of clear liquid. The fact that only the transforming cells present this appearance, while all the surrounding cells are normal, would preclude the possibility that the condition is an artif act. Both nuclei and cytoplasm become more densely staining as further contraction takes place and darkly staining spherules appear in the cytoplasm and in the nucleus. The reduction in volume affects both the cytoplasm and the nucleus.

If changes in volume of the cell, the appearance of spherules in both the cytoplasm and the nucleus and the acquisition of the capacity to take a denser stain may be considered criteria for metabolic activity, the cells have undergone a marked change in their metabolism.

4. *Spermatogonia*

It is impossible to determine the exact number of generations through which the spermatogonia pass before spermatocytes are produced, but it is evident that there are at least five or six. As each spermatogonium gives rise to a group of descendants they form a cyst and all pass through the same stages of division at the same time (fig. 25). Consequently, it is possible to estimate the approximate number of descendants to which a single spermatogonium has given rise by counting the number contained in a cyst.

In the dividing spermatogonium the chromosomes are so massed as to preclude a definite count. The most favorable cells were those prepared in smears, stained with iron haematoxylin and viewed from the pole. The number found in the dividing primordial germ cells, twenty-seven, would probably appear if the chromosomes could be separated so as to permit a count. Dividing spermatogonia are shown in figure 39.

5. *Synapsis and maturation divisions*

There is no growth period after the spermatogonia are formed. In figure 3 all cells have been drawn to the same scale and there is a decrease in size from the largest germ cell to the mature spermatozoon. In this respect the perch differs from the amphibia, from the dipnoans where the primary spermatocytes are described as nearly three times as large as the spermatogonia, and from many other vertebrates where there is a considerable growth. A condition somewhat similar to that in the perch is found in some of the insects.

Some entire cysts show nuclei in which the contents are distributed as slender threads. In many of them the threads are equally distributed (fig. 26), in others there is a contraction stage in which the threads have been drawn into a 'bouquet' at one pole (fig. 40, *a*), while in still other stages there is the last degree of contraction. Here about three-fourths of the nuclei is clear, while the remaining fourth contains the threads drawn together into a dense mass (fig. 40, *b*).

The small size of the spermatocytes and the tenacity with which the chromosomes adhere to each other make any detailed study of the maturation divisions impossible. Figure 41, *a* and *c*, represent a polar and an equatorial view of a primary spermatocyte division in a metaphase. Figure 41, *b*, is an anaphase of the same division. No data were collected which would point toward the presence of a sex chromosome. A large number of dividing spermatocytes were examined, especially in the metaphase and the anaphase, but no lagging chromosomes were observed nor any chromosome proceeding toward the pole more rapidly than the general mass.

6. *Spermatids*

The period in which spermatids are present in any one cyst, like the duration of the spermatocytes in any one cyst, is very short. The entire period in which they may be found in the testis, however, lasts from early September to the middle of December.

After the last division the chromatin collects at one side of the nucleus. There is a gradual reduction in size accompanied by a denser accumulation of chromatin at one side of the nucleus (fig. 42). The nucleus stains more darkly with each stage of progress toward the mature spermatozoon.

7. *Spermatozoa*

The mature spermatozoon has a blunt, kidney-shaped head which stains an intense black with iron-haematoxylin, a small, lightly staining middle piece and a short tail (fig. 43). The spermatids of a single cyst, while transforming into spermatozoa, collect into masses which resemble parachutes (fig. 44). The heads all point in the same direction while the tails are drawn together. As the mature spermatozoa are formed the parachutes become more compact, and in fixed material they may be teased apart without losing their shape. These structures are probably comparable to the spermatophores described in some fishes.

The first spermatozoa are formed about September 10th and are present until their expulsion takes place the following spring. The expulsion is not complete and a few scattered spermatozoa are still to be found in the testis during the early summer months.

V. DISCUSSION OF FACTORS INFLUENCING CYCLE

The three critical points in the variation of the testis, volumetrically, are indicated in figure 1A. The first occurs in the latter part of August when the sudden increase in the size of the testis is started. The second is the beginning of the reduction in size which occurs about November 1, and the third occurs about the middle of March when the volume suddenly begins to drop.

In figure 4 an attempt is made to correlate the internal processes with the curve in figure 1A. Undoubtedly the tremendous increase in the volume of the testis is contemporaneous with the formation of the spermatogonia and the rapid subsequent divisions. While the maturation divisions are taking place new

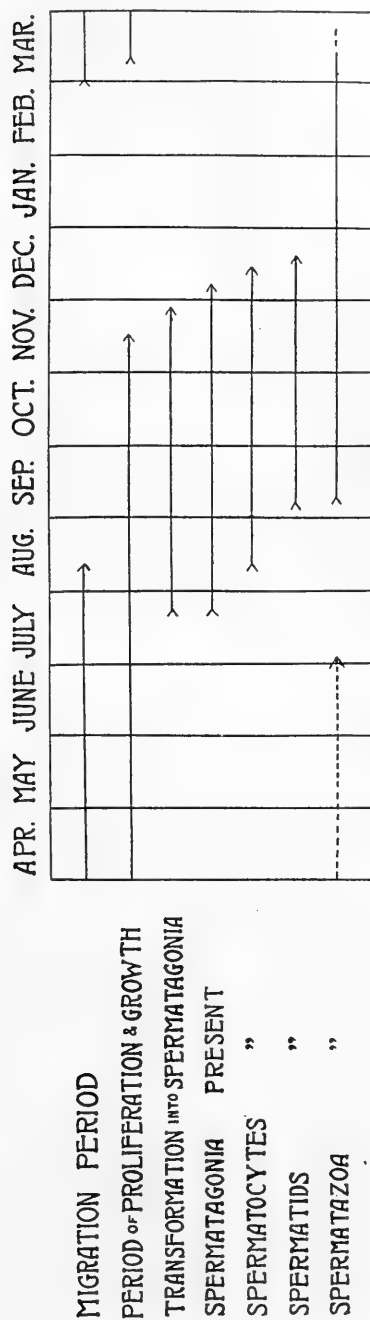


Fig. 4 Drawing illustrating duration of stages in maturation of germ cells.

cells are starting through the procession of changes and divisions which occur before mature spermatozoa are formed. The migration of new germ cells into the testis ceases about the first or the middle of August. The end of this period of migration may or may not have something to do with the decline in the volume of the testis which starts at the same time. It is certain that the sharp decline occurring from March to June is caused by the expulsion of the spermatozoa.

It is not satisfactory merely to point out internal changes in the testis and to correlate them with periodical variations in the volume of the testis unless it be argued that the germ-cell cycle is an automatic and inherent one which will take place independently of the factors of external environment. The very fact that there are seasonal changes justifies a closer scrutiny of the seasonal environment of the fish.

The perch is a bottom dweller. A gill net suspended in the water a few feet above the bottom will trap only a few fish, while one suspended at the bottom in the same locality will trap great numbers. It has been found by Prof. A. S. Pearse that the perch occur in the greatest numbers in Lake Mendota at the bottom of the lake at a depth of from 20 to 35 feet. The records of the Wisconsin Geological and Natural History Survey show that the variation in the temperature between the surface and the bottom of the lake at any given date is a matter of only two or three degrees. The variation in the temperature at the bottom of Lake Mendota at a depth of 6 meters is shown graphically in figure 1*B*. The curve is based on the mean temperature for the last fifteen years at a depth of 6 meters. The base line represents 0 degrees, centigrade, and each space an interval of 5 degrees. The spaces between the ordinates represent intervals of thirty days. During the winter the temperature at the bottom remains slightly above zero. During the latter part of March there is a rise in temperature, and the rise continues till about the third week in August. It will be seen at a glance that the two critical points are early in the spring and late in August and a comparison of these two periods with the critical points on the curve in figure 1*a* shows a coincidence in the criti-

cal points of the two curves. It cannot be positively affirmed from this evidence that changes in temperature are responsible for the initiation of certain processes in the testis, but it is significant that the tremendous synthesis of material which takes place in the testis is started in late August, i.e., in the period in which the temperature of the water surrounding the perch has reached its highest point and has begun to decline, and that the expulsion of spermatozoa takes place at the precise time in which the temperature of the water is beginning to rise. It has long been known at the University of Wisconsin that the date of the spawning of the cisco (*Coregonus artedii*) may be predicted rather accurately by following the temperature of the lake. During the fall of 1916 the temperature of the lake was a little higher than usual and numbers of ciscos were found by Mr. A. R. Cahn to be resorbing their eggs. It seems reasonable to presume that changes in temperature may influence the reproductive processes of the male perch when the reproductive processes in the female cisco are dependent upon such delicate changes in temperature.

SUMMARY

1. A great seasonal variation exists in both volume and in the internal processes in the testis of the perch. The minimum size occurs from late June to late August. The maximum size is attained early in November.
2. The testis is divided by connective-tissue partitions into lobules, but there are no seminiferous tubules.
3. There is a cord of germ cells outside the body of the testis from which the testis is periodically supplied.
4. An active migration of germ cells occurs from the cord, outside of the testis, to the ends of the lobules at the periphery of the testis.
5. The lobules of the testis are gradually filled with an accumulation of germ cells. Transformation of the germ cells into spermatogonia and the process of spermatogenesis take place immediately after the accumulation has filled the lobules.

6. During spermatogenesis the lobules are partitioned into cysts resembling those found in the testis of insects.

7. There is no period of growth after the spermatogonia are formed.

8. The diploid number of chromosomes is 27.

9. The beginning of the period of spermatogenesis is contemporaneous with the beginning of the seasonal reduction of the temperature of the water in which the perch is found.

10. The expulsion of the spermatozoa occurs at the same time as the seasonal rise in the temperature of the water. The beginning of the sudden increase in the size of the testis is simultaneous with the beginning of the seasonal drop in the temperature of the water in which the perch is found.

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PLATE 1

EXPLANATION OF FIGURES

Figures 5 to 22 inclusive, represent a series of camera lucida drawings showing the volumetric increase and decline during the different seasons. The series also represents several variations in form.

- 5 and 6 Average size of testis on July 30.
- 7 and 8 Average size of testis on August 30.
- 9 and 10 Average size of testis on September 25.
- 11 and 12 Average size of testis on October 13.
- 13 and 14 Average size of testis on October 21.
- 15 and 16 Average size of testis on November 20.
- 17 and 18 Average size of testis on January 5.
- 19 and 20 Average size of testis on March 30.
- 21 Average size of testis on April 20.
- 22 Average size of testis on May 5.

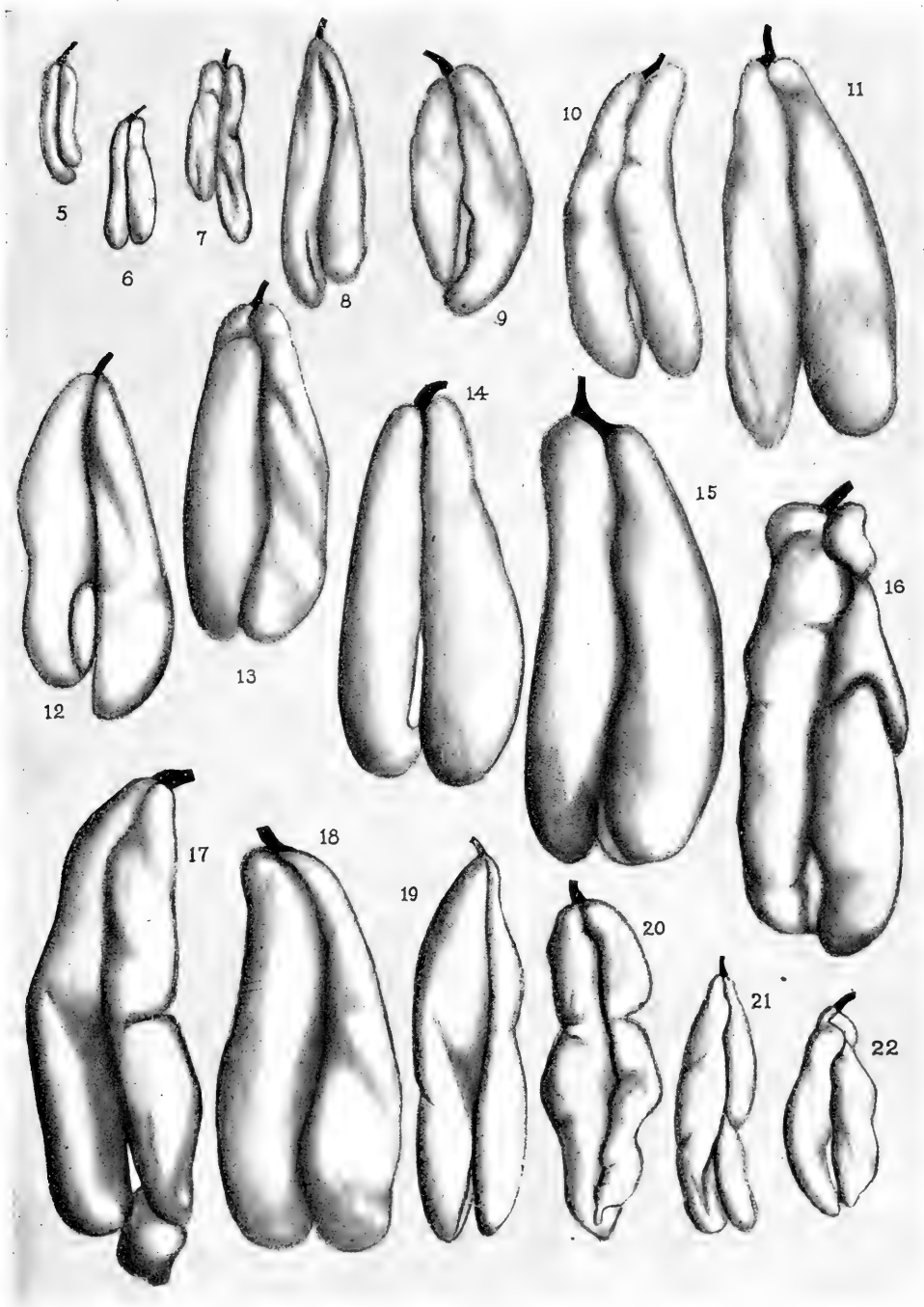


PLATE 2

EXPLANATION OF FIGURES

23 Section of pickerel testis near periphery. *Con.tis.nuc.*, connective tissue nucleus; *g.c.*, germ cells; *lob.*, lobule; *sp.m.*, sperm mass.

24 Section representing part of a lobule in sun fish testis. *con.tis.nuc.*, connective tissue nucleus; *g.c.*, germ cells; *spg.c.*, developing cyst of spermatogonia.

25 Camera lucida drawing of a single lobule of a perch testis during period of transformation. $\times 680$. *g.c.*, germ cells; *div.g.c.*, dividing germ cells; *trans.g.c.*, transforming germ cells; *spg.*, spermatogonia; *div.spg.*, dividing spermatogonia; *lob.w.*, wall of lobule.

26 Camera lucida drawing of peripheral portion of perch testis. $\times 880$. Material fixed November 15. *prim.g.c.*, primitive germ cells; *spg.*, spermatogonia; *div.spg.*, dividing spermatogonia; *sp.td.*, spermatid; *spz.*, spermatozoa; *syn.*, nuclei during contraction stage of synapsis; *tes.wall*, testis wall.

27 Camera lucida drawing of a portion of germ cell cord. $\times 567$. *g.c.*, germ cells; *con.tis.*, connective tissue.

28 Camera lucida drawing of peripheral portion of perch testis. $\times 680$. Material fixed July 3. *bl.c.*, blood cells; *bl.v.*, blood vessel; *con.tis.nuc.*, connective tissue nucleus; *con.tis.fib.*, connective tissue fiber; *lob.w.*, wall of lobule; *m.g.c.*, migrating germ cell; *s.g.c.*, stationary germ cell; *tes.wall*, testis wall; *spr.sp.*, space formerly occupied by spermatozoa.

29 Camera lucida drawing of section of peripheral portion of perch testis $\times 680$. Material fixed May 5. *m.g.c.*, migrating germ cell; *s.g.c.*, stationary germ cell; *bl.cell*, blood cells; *elas.fib.*, elastic fiber in testis wall.

30 Camera lucida drawing of section of perch testis at periphery. $\times 680$. Material fixed August 5. *ad.t.*, adipose tissue; *bl.v.*, blood vessel; *g.c.*, germ cells; *lob.wa.*, wall of lobule; *tes.w.*, wall of testis. (Note: figures 31 to 42 are drawn to the same scale.)

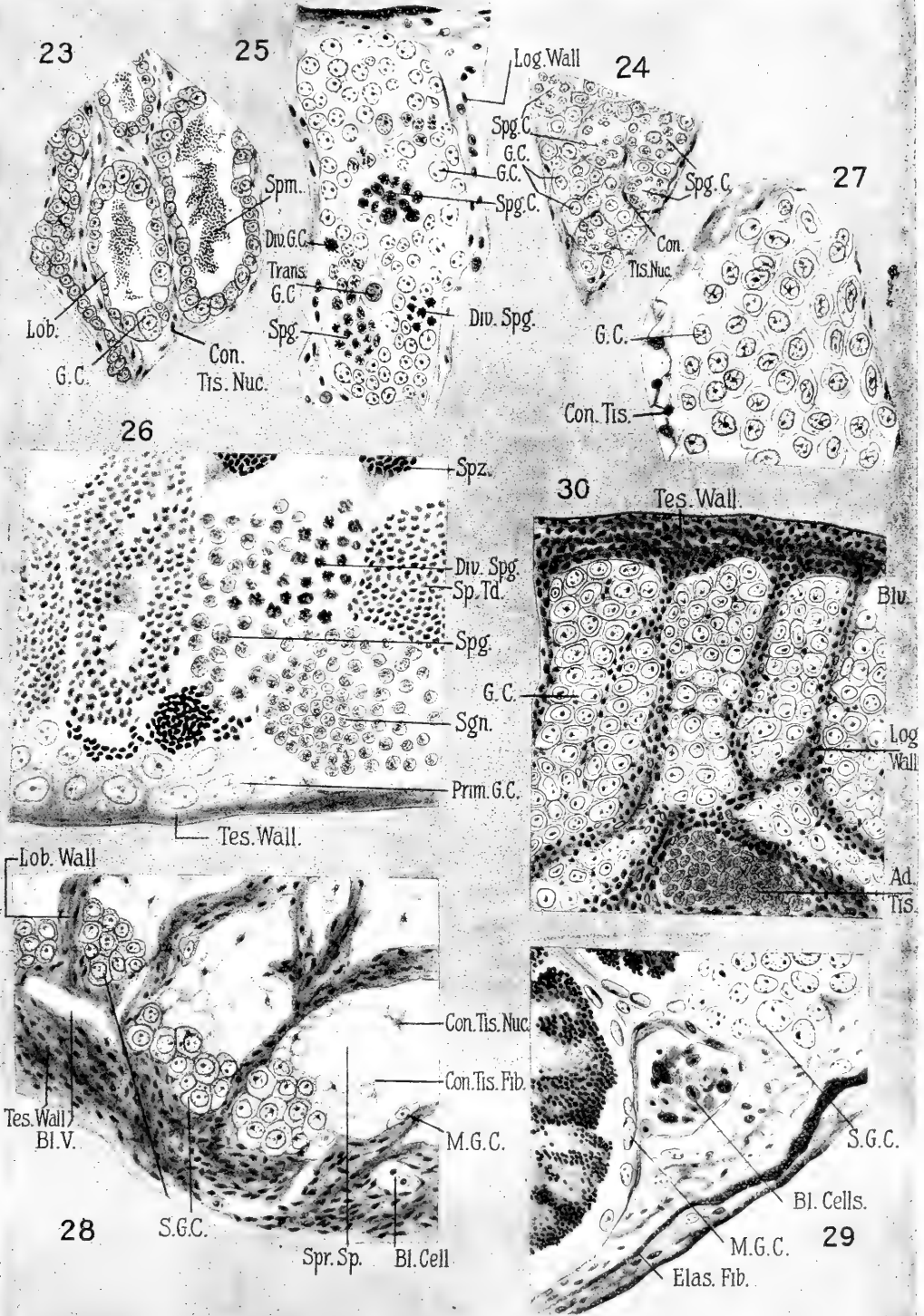
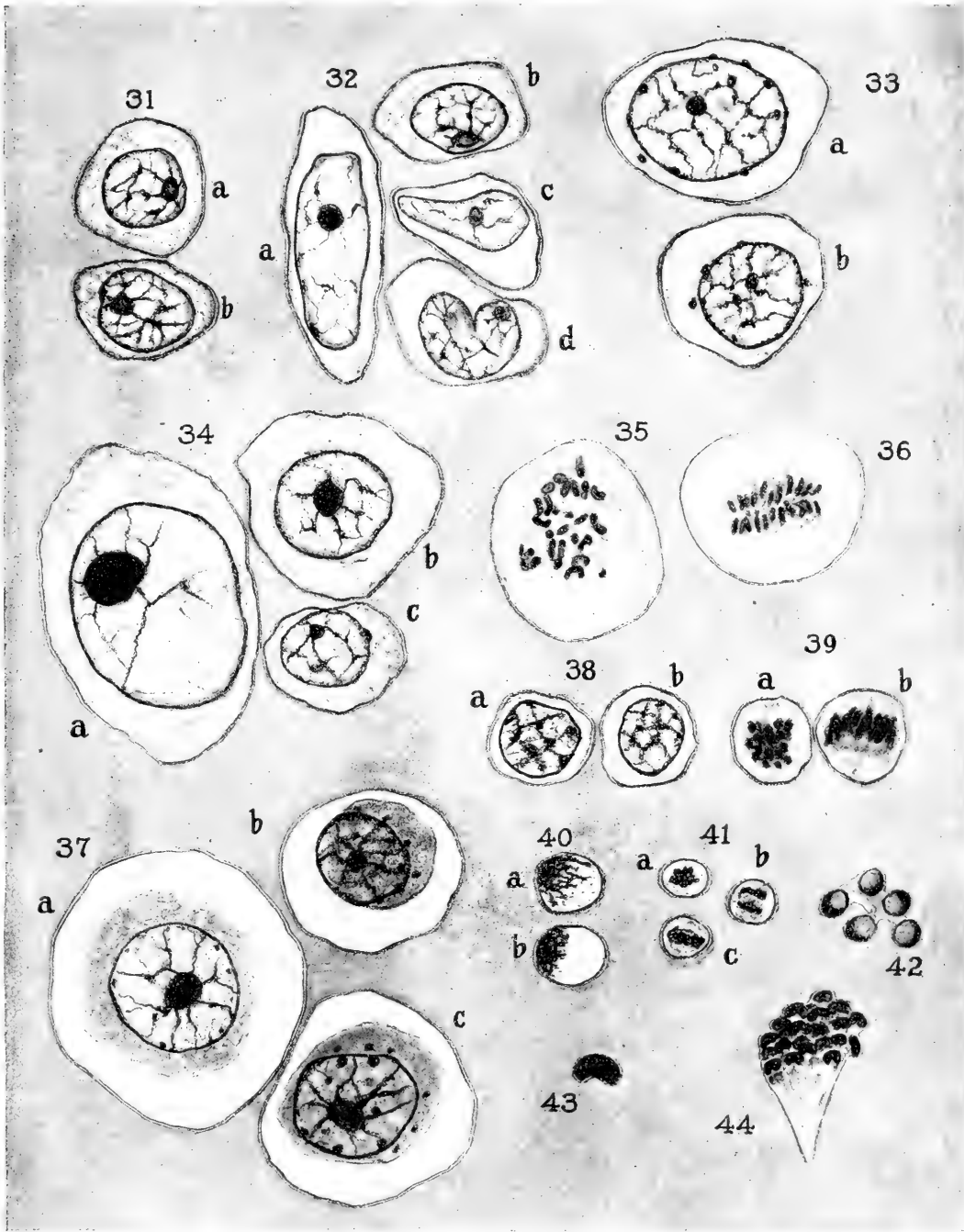


PLATE 3

EXPLANATION OF FIGURES

- 31 *a* and *b*. Germ cells in cord outside of testis before period of migration.
- 32 *a*, *b*, *c* and *d*. Shapes assumed by germ cells during migration to periphery of testis.
- 33 *a* and *b*. Germ cells located at the periphery of testis just after period of migration.
- 34 Germ cells during the period of growth and proliferation. *a*, maximum size; *b*, intermediate size; *c*, minimum size.
- 35 Polar view of dividing germ cell showing 27 chromosomes.
- 36 Equatorial view of early anaphase of dividing germ cell.
- 37 Three stages in the transformation of the germ cells into spermatogonia *a*, early stage; *b*, late stage; *c*, intermediate stage.
- 38 *a* and *b*. Resting spermatogonia.
- 39 Dividing spermatogonia. *a*., polar view of metaphase; *b*, equatorial view of metaphase.
- 40 *a* and *b*. Two nuclei in the bouquet stage of synezeisis.
- 41 Dividing spermatocytes. *a*, polar view of a metaphase; *b*, equatorial view of an anaphase; *c*, equatorial view of a metaphase.
- 42 Transforming spermatids.
- 43 Single spermatozoon greatly enlarged.
- 44 Group of spermatozoa.

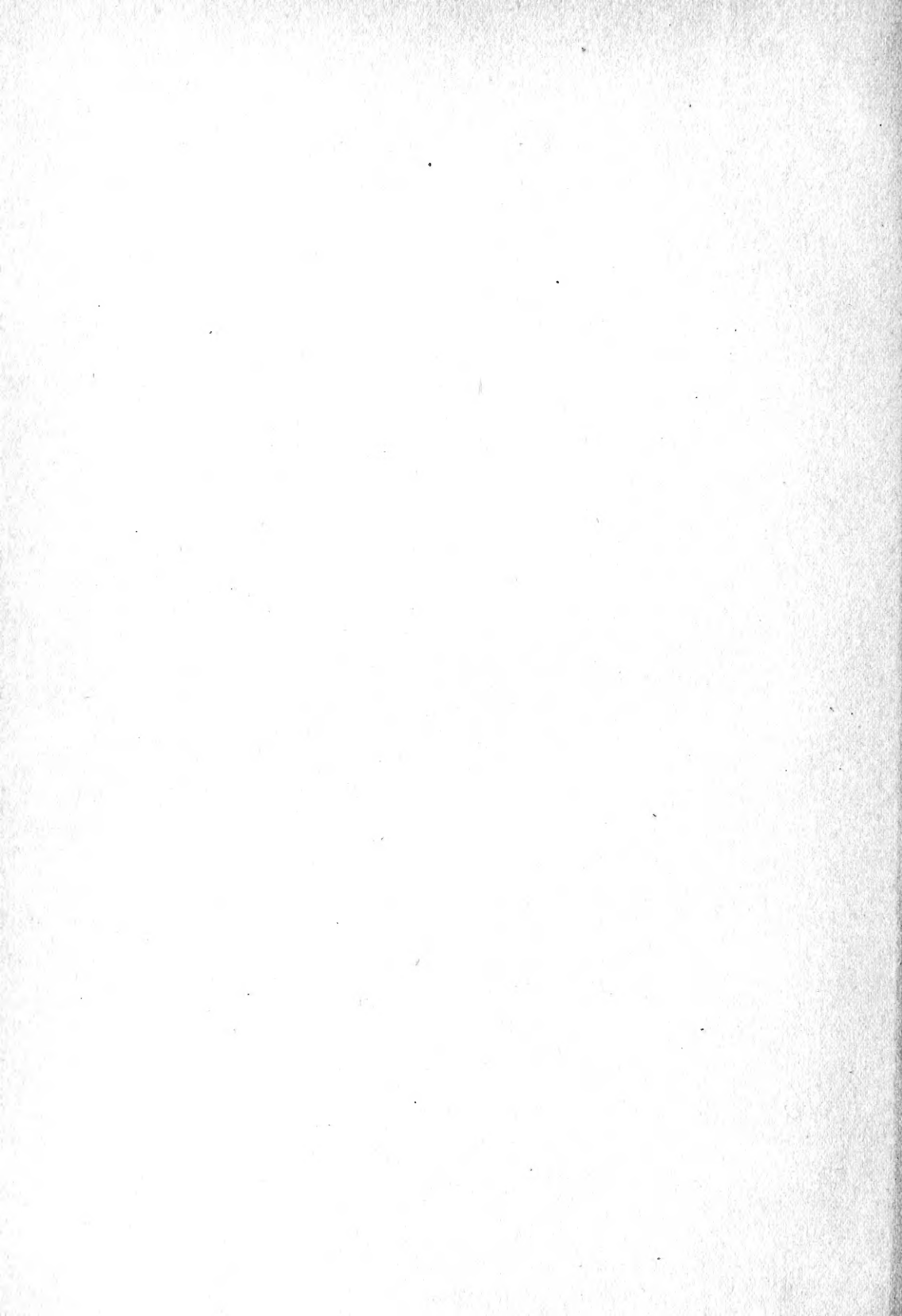




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